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THE JOURNAL OF DENTAL RESEARCH

(Continuing the Journal of the Allied Dental Societies)

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Important Books Received

Rickets including osteomalacia and tetany. By Alfred F. Hess, M.D., Clinical Professor of Pediatrics, University and Bellevue Hospital Medical College, New York City. 1929: pp. 485; 7¼ x 4 in.; 52 illustrations; $5.50. Lea and Febiger, Philadelphia, Pa.

Synopsis of the practice of preventive medicine as applied in the basic medical sciences and clinical instruction at the Harvard Medical School. By the Faculty of Medicine. 1929: pp. 194; 6½ x 3¾ in.; no illustrations. Harvard University Press, Cambridge, Mass.

La pratique stomatologique. IV. Dentisterie opératoire. By Pol Nespoulous, D.D.S. (College dentaire de Chicago), Professor à l'Ecole Francaise de Stomatologie; Stomatologiste des Hôpitaux de Paris. 1929: pp. 541; 6¾ x 4 in.; 263 illustrations; 65 fr. Masson et Cie, Paris, France.

Ueber die Natur der Muskelrheumatischen (myalgischen) Schmerzsymptome. Dr. Folke Lindstedt, Stockholm, Sweden. Supplement XXX of *Acta Medica Scandinavica*, lxxii, 1929: pp. 180; 7 x 4 in.; 13 illustrations.

Testicular grafting from ape to man. Serge Voronoff, Director of the Laboratory of Experimental Surgery of the Collège de France and Assistant Director of the Laboratory of General Biology of the Ecole des Hautes Études; and George Alexandrescu, formerly assistant at the Institut d'Histologie, and Staff Hospital Physician, Bucharest, Roumania. *Translated* by Theodore C. Merrill, M.D. 1929: pp. 125; 6½ x 3¾ in.; 39 illustrations. Brentano's Ltd., London, England.

The properties and uses of drugs. By Henry H. Rusby, Ph.M., M.D., D.Sc., Dean and Professor of Materia Medica, College of Pharmacy, Columbia University; A. Richard Bliss, Jr., A.M., Phar. D., M.D., Dean of the School of Pharmacy, and Chief of the Division of Pharmacology and Materia Medica, Colleges of Medicine and Dentistry and School of Pharmacy, University of Tennessee; and Charles W. Ballard, A.M., Phar. D., Ph.D., Associate Professor of Materia Medica and Director of the Microscopical Laboratory, College of Pharmacy, Columbia University. 1930: pp. 823; 7¾ x 4¾ in.; 38 illustrations; $6.50. P. Blakiston's Son and Co., Philadelphia.

American illustrated medical dictionary. A complete dictionary of the terms used in medicine, surgery, dentistry, pharmacy, chemistry, nursing, veterinary science, biology, medical biography, etc. By W. A. Newman Dorland, A.M., M.D., F.A.C.S., member of the Committee on Nomenclature and Classification of Diseases, American Medical Association, and Editor of the "American Pocket Medical Dictionary"; with the collaboration of E. C. L. Miller, M.D., Professor of Bacteriology and Biochemistry, Medical College of Virginia. *Fifteenth edition.* 1929: pp. 1427; 8¼ x 4¾ in.; 525 illustrations; flexible binding—plain, $7.00; thumb index, $7.50. W. B. Saunders Company, Philadelphia and London.

Dental bibliography. A reference index to the literature of dental science and art as found in the Libraries of the New York Academy of Medicine and Bernhard Wolf Weinberger. Compiled by Bernhard Wolf Weinberger, D.D.S., Librarian of the First District Dental Society; Advisory Librarian, Dental Library, New York Academy of Medicine. *Second edition.* 1929: pp. 183; 7¼ x 4¾ in.; no illustrations. Published under the auspices of the First District Dental Society of the State of New York, New York City.

Deutsche Zahnheilkunde. Heft 75. 1929: 8 x 4¾ in.; mar. s 3.60. Georg Thieme, Leipsig, Germany. (1) Submaxillare Röntgenaufnahmen, ein neues Verfahren zur röntgenographischen Darstellung des Unterkiefers und seiner Zahne. By Dr. J. v. Reckow, Marburg. Pp. 15; 30 illustrations. (2) Klinik und Diagnostik der Osteodystrophia fibrosa localisata der Kiefer. By Dr. Karl Spring, Vienna. Pp. 15; 7 illustrations.

Fundamentals of pathology. By Joseph Schroff, B.S., M.D., D.D.S., Associate Professor of Oral Pathology, School of Dental and Oral Surgery, Columbia University. 1929: pp. 109; 7¼ x 4¾ in.; 40 illustrations; $2.50. William Albert Broder, New York City.

Outline of bacteriology. By Henry A. Bartels, B.S., D.D.S., Lecturer on Bacteriology, School of Oral Hygiene, Columbia University. 1929: pp. 128; 6¾ x 4 in.; 47 illustrations; $2.00. William Albert Broder, New York City.

Annual report (reprints) of the investigations carried out under the supervision of The Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association. *Volume XVII.* 1928: 16 reprints; 7¼ x 4¾ in.; 43 illustrations. American Medical Association, Chicago, Illinois.

Dental histology and embryology. By B. Orban, M.D., Professor of Dental Histology and Pathology, Chicago College of Dental Surgery, Dental Department of Loyola University. *Second edition.* 1929: pp. 218; 7 x 4¾ in.; 201 illustrations; $4.00. P. Blakiston's Son and Co., Inc., Philadelphia.

Four lectures on "local analgesia in dental practice." Delivered, under the auspices of the Dental Board of the United Kingdom, by C. H. Howkins, F. N. Doubleday, F. St. J. Steadman, and A. T. Pitts. 1929: pp. 119; 7¼ x 4¾ in.; 104 illustrations. Dental Board of the United Kingdom, 44 Hallam Street, Portland Place, London, W. I.

Practical conclusions from scientific research in denture construction. By Alfred Gysi, D.D.S., M.D. (hon.), D.M.D. (hon.), Professor of Prosthetic Dentistry and Director of the Histological Laboratory, University of Zurich, Switzerland. 1929: pp. 40; 7¼ x 4¾ in.; 37 illustrations. Dentists' Supply Company of New York.

The rise and fall of oral hygiene in Bridgeport. By George Wood Clapp, D.D.S., Editor of *Dental Digest.* 1929: pp. 104; 8 x 4¾ in.; 11 illustrations. Dental Digest, New York City.

The business side of dentistry. By Edwin N. Kent, D.M.D., Lecturer on conduct of practice, Harvard University Dental School. 1929: pp. 180; 6¾ x 4 in.; 13 illustrations; $5.00. C. V. Mosby Co., St. Louis, Mo.

Treatise on a neglected phase of dentistry. By Henry F. Libby, D.M.D. and Arthur A. Libby, D.M.D., Instructors in dental research, Harvard Dental School; Clinicians in exo-orthodontia, Forsyth Dental Infirmary, Boston, Mass. 1927: pp. 90; 7¼ x 5 in.; 85 illustrations. Privately printed by the authors, Boston, Mass.

A text-book of oral pathology for students and practitioners of dentistry. By Russell W. Bunting, D.D.S., D.D.Sc., Professor of Dental Histology and Pathology, University of Michigan, Ann Arbor, Michigan. 1929: pp. 495; 7¼ x 4¾ in.; 327 illustrations; $7.00. Lea and Febiger, Philadelphia, Pa.

Hygiene of the mouth and teeth. By Thaddeus P. Hyatt, D.D.S., F.A.C.D., Professor of Preventive Dentistry, New York University Dental School, and Assistant Medical Director, Metropolitan Life Insurance Company. 1929: pp. 64; 4¼ x 2¾ in.; 1 illustration. Brooklyn Dental Publishing Company, Brooklyn, N. Y.

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"DENTISTRY" AND "STOMATOLOGY"

This *Journal* uses the term "dentistry" to signify the science and art of oral health-service in all its local and systemic relationships. The meanings of words, which are not necessarily restricted to *etymological* significance, acquire expanded or new import in accordance with convenience and usage. Thus the term "medicine," which originally meant to remedy or cure, now also means *to make remedial or curative treatment unnecessary.* "Stomatology" is a synonym for "dentistry." We prefer the older term because its use respects both the history of dentistry and the efforts of those who have brought dentistry to its present status.

ENDOWMENT FUND OF THE JOURNAL OF DENTAL RESEARCH

The American College of Dentists has appointed a Commission on Journalism and is now giving attention to the Commission's report on dental journalism. While this matter is under consideration, and until decisions are announced, it has seemed desirable to suspend efforts to increase the Endowment Fund, lest any such activity should appear to be related to the endeavor of the College to discontinue trade-house control of journals that are issued as professional magazines. The annual deficit, larger than usual because of the increased bulk of this Volume, will be liquidated as heretofore by supporters of the cause of professional journalism.

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The dental societies named on the title page publish their proceedings on conditions that make each society's group of papers, in effect, its own independent journal within the *Journal of Dental Research.* Papers contributed by societies are controlled and edited by the respective associations through their official representatives. Reprints of parts of any number may be obtained for issuance in separate volumes of transactions. See the title page of this Volume, and compare it with those of Volumes V–IX.

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May 16, 1929

U. G. RICKERT, A.M., D.D.S., *Editor*

Joint meeting of the Ann Arbor Section of the International Association for Dental Research, and the First District Dental Society of the State of Michigan, of Detroit. Sessions held at the School of Dentistry, and at the Michigan Union, University of Michigan, Ann Arbor, May 16, 1929. Chairmen: the presidents of the two organizations concerned, Drs. M. L. Ward and W. J. Whiteman, respectively. There were afternoon and evening sessions, and a banquet between.

Scientific program

1. *Afternoon session: informal; Metallurgical Laboratories of the Dental School*
   I. Physical studies of clasp metals. *M. L. Ward*, D.D.Sc., and *E. O. Scott*, M.S.. 1
2. *Evening session: following the banquet; Michigan Union*
   II. News from the sky. *W. C. Rufus*, M.A., Ph.D..... 4

Abstracts

I. PHYSICAL STUDIES OF CLASP METALS

Marcus L. Ward, D.D.Sc., and E. O. Scott, M.S., School of Dentistry, University of Michigan, Ann Arbor, Mich.

As no work had been done on the precious metals and their ability to resist alternating stresses, it was necessary to develop a machine to test the fatigue under these stresses. While there was little or nothing to serve as a guide in the construction of a machine with which to apply alternating stresses to clasp metals, practically every type in present use had been studied in an effort to develop one that would duplicate as nearly as possible the stresses to which clasps are sub-

[1] The last preceding series of abstracts of the proceedings of the Ann Arbor Section was published in the *Journal of Dental Research*: 1929, ix, p. 75.

jected when attached to partial dentures. Results with this type of machine over a period of two years, together with clinical observations for several years, made it appear that many of the failures in partial dentures and removable bridgework, to which clasps had been attached, were due to faulty formulae and to lack of knowledge of the ability of these metals to resist fatigue from alternating stresses.

An exhibit was made of the broken ends of several well known and widely-sold clasp metals. The analytic results show that some of them had been constructed with high precious-metal content, and heralded and advertised as desirable for use in the construction of clasps because of the high precious-metal content. The analyses also showed that others were composed of as many as seven metals, when it is well known that the simplest alloys are more uniform in their behavior. In a few, nickel and zinc had been substituted for platinum, with the result that, when an attempt was made to re-melt one of them, there was a high percentage of loss of material, and an occlusion and perhaps solution of oxides in the interior, which caused them to break easily when subjected to flexure. A number were so constructed as to cast easily; that is, they were quite fluid when molten, in contrast to others containing a higher percentage of platinum and having a thicker consistency.

Numerous other variations of these main classes of alloys, which were apparently sold on the basis of hardness tests, were found in products on the market. All but two of these alloys, when cast into small bars 25 mm. in length, 1 mm. in thickness, and 2 mm. in width, and subjected to flexure tests within each one's range of elasticity without permanent deformation, were found to be of little practical value. Two, which were composed of only four metals—gold, silver, copper, and platinum—showed possibilities that the others did not, and work was begun immediately to determine the conditions under which the best results could be obtained with them. Several years of experience, supported by the literature on the subject, made it seem inadvisable to do much work with the alloys containing more than four metals. This class of alloys had invariably shown occlusion of oxides and gases at the broken ends, aggregates of what appeared to be single metals not properly alloyed, and large facets which made them easily broken when subjected to any kind of a flexure test.

In the development of a technique for the manipulation of the two best alloys, further attention was given to the pressure in casting. Dentists generally have been concerned about the production of fine margins and accurate castings for fillings, but little general information has been given, if it existed, about conditions for the production of castings to be subjected to flexure. Illustrations of castings made with low pressure caused by a small number of revolutions of a centrifugal machine, as compared with those made with a larger number of revolutions, showed a marked difference in the hardness of a given casting at points remote from the sprue and also at the sprue, where the pressure had been high in the former instance and low in the latter. Illustrations were also presented to show that, when a given number of revolutions was maintained and the quantity of gold increased, practically the same thing occurred. These illustrations showed plainly under observations with a microscope, with hardness tests, and with flexure tests. When the work was begun, the number of flexures at which one of these bars of clasp metal would break was from 10 to 600. With the improvements in casting, these two clasp metals resisted from 80,000 to 100,000 flexures. The microscope, hardness-testing equipment, and flexure tests, all seemed to confirm one of our early observations, namely: that pressure was capable of varying the structure of gold and, as a result of this variation, flexure, hardness, and elasticity are changed or varied.

As the work progressed with these two clasp metals, it was found extremely important to have the crucible, in which the gold was melted for casting, hot and everything in readiness so that the quickest possible melting of the metal might be made. With these improvements, the number of fractures showing the cleavage lines owing to occluded oxides was materially lessened. The broken ends of some of these, which had consumed two or three minutes to melt, were shown.

Another interesting feature was the heat treatment of the specimens. During the early part of the work on these two clasp metals, it was suspicioned that the rate of cooling, after making the casting, affected these alloys as heat affects most other alloys. Practically every well known technique for cooling the casting was tried, with results varying so much that it was difficult in many cases to tell what had happened. We then decided to connect with our experimental

furnace a rheostat that would hold the temperature anywhere desired, and heat-treat these specimens for given periods, and then plunge them. We began by heat-treating them at 750°C. for from 15 minutes to 2 hours. The temperature was first reduced to 450°C. with time maintained. Later, the reverse was tried with the temperature the same and the time varied. We find that 450°C., held for 2 hours and then the specimen immediately chilled, allows us to subject these specimens to fatigue at an average of over 1,000,000 times before breaking. Experiments are now being conducted to determine the exact point at which one of these metals (Ney G3) will yield a maximum number of flexures. A detailed report of the completed work will be published.

II. NEWS FROM THE SKY

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News from the sky comes on the wings of light, the fastest thing in the universe. From the moon it takes 1⅓ seconds; from the sun, 8⅓ minutes; from the nearest star, Proxima Centauri, 4⅓ years. An aeroplane, at 100 miles per hour, would take a hundred years to the sun and 30,000,000 years to the star.

Step on the light: a nine years' trip brings us to Sirius, the brightest star in the sky as seen from the earth. One hundred years ago astronomers found that it deviated from the straight and narrow path computed for its motion, and Bessel concluded that its wayward course was due to a dark companion. Several years later, Alvin G. Clarke, testing a telescope lens, detected the faint object, which has only 1/10,000 the luminosity of the bright star, but nearly ½ its mass. Its spectrum shows that it is a white-hot star. Here was a dilemma. Its faintness argued a small size or a low surface luminosity. Its mass seemed to contradict the first, its heat denied the second. The only way out was a density far transcending any known substance, terrestrial or celestial. Theory gave the white dwarf, as it is called, a small diameter with a density 50,000 times as great as water. One gallon of the star stuff would weigh 200 tons.

Here was a new opportunity to test Einstein's theory which predicts

that the vibrations of the electrons within the atoms producing light will be slower in a strong gravitational field; so the spectrum of the white dwarf was questioned and the size, density and theory agreed. Stranger still, this star material, so dense that a cupful would be a truck load, is not a solid or a liquid, but possesses the properties of a perfect gas. The accepted explanation is that the atoms are stripped of their outer electrons, which take up the greater part of the atomic space, leaving the nuclei nearly bare, which provide by far the largest share of the mass.

On we go on the wings of light, 100 years, passing comparatively few stars. 200 years and we come to Betelgeuze, a giant red star in Orion, as luminous as 1200 suns. Its diameter, measured by Michelson, is 240,000,000 miles. So immense it is that the sun could be placed at its center, around the sun the orbits of Mercury, Venus and the Earth would be far within the giant star, while distant Mars would circle near its surface. This inflated, bubble of gas, moreover, has a density only 1/3,000,000 that of water.

In 600 years we come to Deneb, gem of the Northern Cross, with 10,000 times the light of the sun, and Canopus, queen of the southern skies equal to 80,000 suns. Feature that in the good old summer time. And here is the Orion nebula, three light years across its most luminous part, yet its mass is not more than 10,000 suns. So slight is its density that it is almost absurd—one millionth of one billionth of air. Stranger news from the nebulae recently arrived. Nebulium, a hypothetical element of the nebulae, discovered by its lines in the spectrum, has been identified as ionized oxygen and nitrogen, gases that compose the air we breathe. Here is additional evidence that sun, planets, stars and nebulae are composed of the same material as the earth, proving that the universe is one. Even the relative abundance of the elements in the sun and stars bears a striking resemblance to the structure of the earth.

On and on we go on the wings of light, 1,000 years, 10,000, 100,000, and we are not growing old. Time on a light wave is always zero; we are in the eternal now. Stars are getting scarce, we are near the limit of our sidereal system. We pass the marvelous stellar clusters; we are beyond the milky way. As it recedes, the galactic system with its forty billion stars shrinks to the size and shape of a spiral

nebula. We have reached the region of the spirals, here is one of the nearest.

"Extra! Extra! All about the Andromeda Nebulae. A new star suddenly appeared." Go tell your editor that is no scoop. Tell your reporter that is old stuff. That new star blazed out 900,000 years ago. According to news from the sky there are about a half dozen spiral nebulae within a million light years, i.e., near enough to show star images in their photographs. Beyond this are millions of spiral nebulae that leave faint traces on the photographic plate. And each spiral nebulae, according to present interpretation, is a separate sidereal system similar to our own galaxy with its billions of stars of which our sun is one.

A new telescope with a mirror 200 inches in diameter is now being planned with four times the light-gathering power of the present giant reflector at Mount Wilson. Thirty tons of fused quartz will be necessary for the mirror. With its increased space-penetrating power, additional news may be expected from the sky.

With increased equipment, on and on we go on the wings of light. We cannot stop. Radiant energy changes not its speed. But the celestial policeman holds up his hand. Detour! Space is curved, light bends under gravitation and can never leave the material universe. We have traveled 100,000,000 years to the most distant spiral nebula on the horizon of our consciousness, but the horizon has extended, we are still at the center of the universe. On and on we go as the eternal light-waves lap on the restless shores of elusive time and space. In the meantime, our bodies cling to an infinitesimal speck of dust we call the earth, for an infinitesimal moment of time, our boasted three score years and ten—infinitesimals of the second order in the endless space-time universe of modern science.

BIOCHEMICAL STUDIES OF SALIVARY MUCIN

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I. INTRODUCTION[2]

This series of studies, undertaken at the suggestion of Prof. Gies to extend previous work by Lothrop (22, 23) under his guidance, was intended to provide chemico-physiological data relating to saliva. The experiments were started shortly before Prof. Gies began his study of dental education for the Carnegie Foundation. They have recently been completed under the supervision, and with the co-operation, of Prof. Miller.

Mucins, which belong to the glycoprotein group of albuminous substances, are the chief colloidal constituents of various kinds of mucus, and of such mucigenous secretions as saliva. The physiology and pathology of mucus secretion in general has received comparatively little chemical attention, and the knowledge of salivary mucin in par-

[1] William J. Gies Fellow in Biological Chemistry at Columbia University. First appointee, Feb.–June, 1928. See Miller: *Journal of Dental Research*, 1929, ix, p. 742.

[2] Sections I to IV, inclusive, contain data and observations that were published in the author's Ph.D. dissertation: Columbia University, 1924.

ticular, chemically as well as functionally, is notably incomplete. The elaboration and use of a more accurate method for the quantitative determination of mucin, in saliva, appeared to be a useful effort for the furtherance of the general purpose of this series of studies.

Mucin[3] was first examined by Städeler (37). Various workers studied mucins from widely different sources: Obolensky (29), Hammarsten (10), Lothrop and Gies (23), and Dejust (5) from *salivary glands*; Müller (27) and Lothrop (22) from *saliva*; Hammarsten (12) and Landwehr (17) from bile; Eichwald (6), Cavalcaselle (3), and Hammarsten (9) from *snail*; Holst (13) from *synovial fluid*; Levene (20) from *gastric membrane* and other sources; Giacosa (7) and Schulz and Ditthorn (36) from *frog spawn*; Hammarsten (11) from *perch eggs*; Müller (28) from *mucosa of air passages*; Charrin and Desgrez (4) from *bacterial products*; Ishii (14) and Oshima and Tadokoro (30) from *yams*; Weleminsky (39) from *tubercle bacilli*; Sahlstedt (35) from *horse urine*; Rosenfeld (34) from *Cydonia seeds*; and Bresslau (2) from the *slime on Colpidium*. The carbon, hydrogen, nitrogen, oxygen, sulphur, ash contents, etc., have been determined with varying results, and qualitative reactions have been noted. The hydrolytic products of mucins have been studied by Mörner (26), Müller and Seeman (28), Steudel (38), Otori (31), Malenük (24), Levene (20) and collaborators, and by others.

The relation of salivary mucin to dental caries and oral pathology has been investigated by Lohmann (21), Miller (25), Gies (8), Lothrop and Gies (23), Kirk (16), Pickerill (32), and Jones (15).

II. PREPARATION OF SUBMAXILLARY MUCIN AND MUCINATE

A. Mucin

Mucins, like all proteins, combine with acids and with bases. In secretions, mucins occur in solution chiefly in the form of the protein combined with basic elements or radicals (mucinates), although mucin is often present to some degree in insoluble form, as mucin itself or as "acid salts" of mucin, or both.

[3] In this discussion, the mucoids, including some of the connective tissue proteins previously called mucins, are assumed to be sufficiently unlike the true mucins to justify their omission. Accurate differentiation among the different types of glycoproteins is impossible at present.

The first preparation of submaxillary mucin was made by Städeler (37) in 1859. He cleaned and washed submaxillary glands, ground them with glass, extracted overnight with water, filtered, precipitated the mucin with acetic acid, washed the precipitate with hot alcohol, and dried the product. Obolensky (29) extended this work. In 1881 Landwehr (17) extracted mucin from bile with strong hydrochloric acid, and precipitated it by neutralization or by dilution with water. He then redissolved it, reprecipitated with dilute hydrochloric acid, washed the precipitate several times with alcohol and ether, and dried the product at 110°C. Later, these methods were found to be unsatisfactory.

The method of extracting mucin from tissues with weak alkali has been used by several workers. In 1920 Dejust (5) prepared mucin from submaxillary glands by extraction with 0.5 percent potassium hydroxide, followed by precipitation with acetic acid. In 1888, Hammarsten (10) introduced an improved method, later modified by Levene (18), for the preparation of mucin from submaxillary glands. This method (1), with minor modifications, was adopted for the work described in this paper, as outlined below.

Submaxillary glands of oxen, obtained fresh at a slaughter house, were freed from extraneous tissue, finely hashed, and extracted twice with distilled water overnight, with chloroform as a preservative. The extract was siphoned and filtered through cheesecloth and silk cloth. The filtrate was shaken with ether to extract fat and to favor segregation of particles, and allowed to stand overnight. The mixture was then strained through silk cloth and filtered through paper. The filtrate, viscid and clear, was precipitated with dilute hydrochloric acid—added until the proportion in the mixture was 0.03 percent. The precipitate was redissolved in 0.15 percent hydrochloric acid, the mucin chloride solution strained through silk cloth to remove particles of undissolved mucin, and the dissolved mucin reprecipitated by pouring the solution into distilled water (four volumes), with vigorous stirring. This purification was conducted four times, the mucin separating at each precipitation as characteristic stringy masses, in most cases depositing on the stirring rod, though sometimes floating on the surface as a viscous, gummy mass. The final precipitate, washed by agitation in a large volume of distilled water, was then

treated with freshly distilled neutral alcohol and sedimented. This was repeated several times until the strength of alcohol reached 95 percent. The mixture was then filtered. The product, washed three times with ether, was a white powder, which, dried at room temperature in a vacuum desiccator, darkened very slightly. The yield of purified mucin varied from 4.81 to 7.87 grams per kilogram of fresh hashed gland tissue. It may be noted, in passing, that a 9-pound lot of parotid salivary glands yielded no mucin by this method.

Centrifugation could not be satisfactorily substituted for filtration in the early stages of the method, because of the poor separation of tissue debris, etc., and also because the volumes were large. Purification by re-solution in sodium hydroxide was not as satisfactory as in hydrochloric acid, because the length of the periods of contact with alkali caused denaturing of the mucin. One product, prepared by precipitation from sodium hydroxide solution, contained 13.8 percent of nitrogen, which was identical with the value reported by Landwehr (17). But the product did not have the characteristic properties of mucin. The powder, exceedingly light, was not viscous in water. Upon addition of $N/5$ sodium hydroxide, no heavy slimy viscid sodium mucinate was formed. Its solution gave heavy precipitates with lead acetate (page 12).

B. Mucinate

Water-soluble mucin salts (mucinates) were prepared, from purified mucin products, by the method used by Lothrop (22), with the elimination of 0.1 percent sodium hydroxide solution, as follows:

Small portions of mucin were triturated in a mortar with 100 parts of 0.5 percent sodium *bicarbonate* solution. To the combined mixtures were added 10 volumes of 0.5 percent sodium *carbonate* solution. This mixture was allowed to stand over night at a low temperature. Large swollen jelly-like masses occurred here and there. The liquid, after being squeezed through silk cloth, was a white, thick, paste-like mixture, which when stirred became entirely transparent as the suspended matter completely dissolved. The solution, in a freshly prepared collodion bag of 2-liter capacity, was then dialyzed against running freshly-distilled water until the solution became acid to

phenolphthalein while it remained alkaline to litmus (pH between 7 and 8). This dialysis required at least eighteen hours. The mucinate solution so obtained was then precipitated by mixture with eight volumes of neutral 95 percent alcohol. When the dialyzed mucinate was first mixed with the alcohol, no appreciable precipitation occurred, even after standing for thirty minutes. However, upon addition of 3 cc. of 20 per cent sodium chloride for every 2250 cc. of the alcohol-water mixture, with stirring, the fluid became opalescent, then milky, and finally a heavy white precipitate settled. The first fraction was collected at the end of three hours of sedimentation; the rest at later intervals. Each of these samples was washed with cold alcohol until free from chloride, then washed thrice with ether, and dried in a desiccator to constant weight.

The product consisting of the first sedimentation (3 hours) was not wholly soluble in distilled water, but required addition of a small amount of sodium hydroxide solution to effect complete solution. The other samples, however, were easily and entirely soluble in distilled water.

These preparations of sodium mucinate contained 10.45 percent of nitrogen. They yielded reducing material on hydrolysis, but no purine base could be detected in the hydrolysate. The sodium content (determined by careful ignition with sulphuric acid) was 1.8 percent. The ash of the purified mucin and mucinate always contained a faint trace of iron.

Through the courtesy of Prof. Gies, two samples of sodium mucinate (VI and VII), prepared by Lothrop (22), were compared with the products made by the method described above.

In the quantitative experiments described below, weighed amounts of mucinate were dissolved in distilled water. Usually, small additions of ether were made to break the foam and to act as a preservative. When necessary, small amounts of sodium hydroxide were added to effect complete solution—Lothrop's products VI and VII required 1.8 cc. and 0.9 cc. of $N/5$ alkali, respectively, in 100 cc. of a 0.1 percent solution of the mucinate. This addition of alkali did not make the solutions alkaline to phenolphthalein. The solutions were kept in a refrigerator, but were brought to 20°C. for pipetting.

III. PRECIPITATION OF MUCIN

A. Conventional methods

A review of the reactions of mucin, as reported in the literature, suggested several possibilities for the separation of mucin from other proteins occurring with it, such as the precipitation of associated proteins by ferrocyanic acid in the presence of sodium chloride, etc. Without giving the details of the negative results, it will be sufficient to say that these methods were not useful for the isolation of mucin.

One very interesting condition was discovered. It is commonly stated that mucin is precipitated by salts of lead and mercury. To our surprise, the addition of lead acetate or mercuric chloride (5 to 10 percent), to solutions of sodium mucinate in concentrations up to 0.1 percent, did not precipitate the mucin. Many such tests were made, and care was taken to keep the reaction of the mixture definitely on the alkaline side of the isoelectric zone of the mucin. In the presence of an excess of alkali, the addition of the metallic salt caused an opalescence, or a discrete precipitate of metallic hydroxide. These mucinate-metallic salt mixtures were filtered, and the nitrogen content of the filtrate was determined. As a rule, all of the mucinate remained in the filtrate. However, in spite of the fact that variable factors such as reaction, time, concentration, etc., were carefully controlled, quantitative recovery of the mucin could not always be effected. Similarly, serum protein, from dog blood or ox serum in a known amount, was added to a standard mucinate solution, the mixture treated with metallic salt, and the liquid filtered. The analytic figures for nitrogen indicated complete separation of the serum protein from the mucinate by the lead salts; incomplete, by the mercury salts.

Attempts were made, with mucinate solution and with human saliva, to precipitate the mucin with hydrochloric acid or acetic acid and to remove the precipitate by filtration for subsequent weighing or nitrogen determination. But only 50 to 80 percent of the mucin could be obtained in this way. At the point of maximum precipitation, from solutions of biological concentration, the mucin separated in the form of a practically permanent turbidity that could not be properly removed either by filtration or centrifugation.

B. Nephelometry

The difficulties indicated above, while ruling out the use of a direct *gravimetric* method for the determination of mucin after acid precipitation, suggested that the conditions were such that *nephelometry* might be adapted accurately. The immediate necessity then became a study of the conditions of this precipitation for the nephelometric determination of the amount of mucin precipitated from saliva by acidification.

We found that, for the precipitation of mucin from solutions of biological concentration, $N/0.02$ hydrochloric acid was a convenient strength. At a pH of from 2.95 to 2.75, titration with this acid gave a maximum turbidity as shown by nephelometric observations. Titration until an additional drop of the acid solution caused no visible increase of turbidity gave an accurate endpoint. The curve of turbidity is flat at this point, and with care there is no danger of adding enough excess of acid to cause diminution of the turbidity by resolution of the mucin.

Similar results were obtained with solutions of our different mucinate products in concentrations of from 0.2 to 0.025 percent. Different portions of the same mucinate solution, so titrated to maximum turbidity, afforded identical nephelometric readings. Any mucinate solution, diluted with water to known volumes and then titrated to maximum turbidity, gave the theoretical reading compared with the original. There were divergent readings for a given solution compared with another preparation in cases where the degrees of purity of the mucinates differed. But the products which were the purest yielded practically identical turbidities.

The first difficulty in applying these results to salivary analysis was the fact that normal saliva is usually turbid owing to the presence of epithelial debris, etc. This initial turbidity must be removed before nephelometry can be applied. Direct filtration or centrifugation of the saliva was tried. When filtered, however, mucin, as it ordinarily occurs in saliva, does not distribute itself evenly between the filtered and the unfiltered portions. Similarly, the lower layer of a centrifuged sample contains a higher proportion of mucin than the upper layer. This partial separation by filtration or centrifugation may

depend upon the condition that some of the protein in the saliva, possibly as mucin or as acid salts of mucin *instead of the normal mucinate*, although highly hydrated and apparently in homogeneous solution, is not as minutely dispersed as the rest.

When saliva or mucinate solution was made decidedly alkaline by the addition of sodium hydroxide, it could then be readily filtered clear, and an aliquot portion of the filtrate contained the theoretical amount of mucin nitrogen. This then was adopted as the means of clearing the saliva of its initial turbidity.

The next difficulty arose from the fact that when this alkaline filtrate was treated with dilute hydrochloric acid, precipitation of the mucin was incomplete. It has long been known (Hammarsten, 9) that the addition of a fairly large amount of sodium chloride (5 to 10 percent) to a mucinate solution may completely prevent the precipitation of the mucin by acid. Dilute solutions of mucinate (0.1 and 0.05 percent), plus sodium chloride (2 *M* solution) in known amounts, were precipitated with hydrochloric acid, and the resultant turbidities compared with that produced in another sample treated in the same way but to which no salt had been added. Small proportions of salt in the precipitation mixtures (as low as 0.05 *M*) caused an appreciable diminution in the turbidity.

The only feasible way to obviate this difficulty was the removal of the salt by dialysis. Therefore the alkalized and filtered saliva or mucinate solution was dialyzed in a collodion bag against running water until acid to phenolphthalein, and then against several changes of distilled water. The dialyzed material could then be made up to known volume, titrated to maximum turbidity with $N/0.02$ hydrochloric acid, and this turbidity compared with a standard of pure mucinate solution so precipitated. The details of this procedure are outlined below. The nephelometric standard may be set conveniently at 20 mm., and the concentration of the unknown calculated in the usual way by inverse ratio of the readings, provided the readings do not differ by more than 20 percent. If the difference in the readings is greater than 20 per cent, another reading must be made, after appropriate dilution of the standard solution or of the unknown, so that the readings will more closely approximate each other.

IV. QUANTITATIVE DETERMINATION OF MUCIN

The analytical method, as adopted, may be outlined as follows: To a 20 cc. portion of saliva, 5 cc. of 5 per cent sodium hydroxide solution are added.[4] The mixture is thoroughly stirred and filtered through dry paper. An aliquot portion (20 cc.) of the filtrate is then placed in a collodion bag (made for convenience in a 100 cc. centrifuge tube), and dialyzed against running water until no longer alkaline to phenolphthalein; then against several changes of distilled water. The time required for this dialysis is usually about five hours. The contents of the bag are then transferred to a volumetric flask of 50 cc. capacity, the washings from the bag added, and the flask is filled to the mark with distilled water. A portion (10 to 20 cc.) of this solution is then treated with $N/0.02$ hydrochloric acid from a burette, drop by drop until a drop fails to increase the turbidity. This is then compared nephelometrically with a standard prepared as follows: 0.1 gm. of sodium mucinate is dissolved in water in a 100 cc. volumetric flask, with the aid of a minimum amount of alkali if necessary; the flask filled to the mark with distilled water; and the excess of alkali dialyzed from a 20 cc. sample, as described above. The contents of the bag are then washed into a 50 cc. volumetric flask, and with distilled water made to volume, of which 10 or 20 cc. are then titrated with $N/0.02$ hydrochloric acid to maximum turbidity. If there is available a mucinate product that is completely soluble in water, without addition of alkali, it may be used directly without dialysis, in a strength of 0.05 percent, as the standard. If the nephelometric readings differ by more than 20 percent, a new dilution of the standard or of the unknown should be made to bring the readings more closely together.

The concentration of mucinate in the saliva may be calculated by the following formula:

$$\text{Mucinate content of saliva} = \frac{\text{Neph. reading of standard}}{\text{Neph. reading of unknown}} \times 3.125 \times \text{concn. of standard}$$

[4] The destructive tendency of this treatment, in view of the denaturing effect of alkali on mucin, is recognized. Our results indicate, however, that, in the concentration of alkali used for the time during which it is in contact with the mucin, no appreciable error is introduced.

For the first example in the accompanying summary, the standard mucinate (after dialysis) was 0.04 percent. In the nephelometer the cup containing the standard was set at 20; the "unknown" gave a reading of 19.7. Therefore the amount of mucinate in the "unknown" was: $\frac{20}{19.7} \times 3.125 \times 0.04 = 0.127$ percent.

Human saliva, the flow stimulated mechanically by mastication of pure paraffin, was analyzed by this method, and the mucin content calculated. Pure sodium mucinate was added to several portions, and the mixtures were analyzed to determine the percentage recovery of the added mucinate. This could usually be made with an error of less than 4 percent. Examples of the analytic results are given below:

Portion of saliva	Mucinate in saliva	Mucinate in saliva	Mucinate added	Total mucin theoret.	Mucinate found	Difference	Loss in recovery
cc.	*percent*	*gram*	*gram*	*gram*	*gram*	*gram*	*percent*
20	0.127	0.0254	0.0050	0.0304	0.0303	0.0001	−2
20	0.146	0.0293	0.0050	0.0343	0.0341	0.0002	−4
25	0.077	0.0192	0.0113	0.0305	0.0302	0.0003	−2.6
20	0.281	0.0562	0.0150	0.0712	0.0708	0.0004	−3.3
20	0.155	0.0310	0.0200	0.0510	0.0508	0.0002	−1
20	0.100	0.0201	0.0200	0.0401	0.0403	0.0002	−1

A further check was made by the addition of small amounts of serum protein. This did not change the analytical results for the mucin.

V. MUCIN (MUCINATE) CONTENT OF SALIVA[5]

The method was used to determine the mucinate content of human saliva. The subjects were "average" adult males, selected without reference to dental conditions. Saliva, *stimulated in its flow* by mastication of pure paraffin (62 samples, 27 subjects) had an average mucinate content of 0.27 percent (calculated as Na mucinate). The "probable error" for the series was 0.06 percent. The range was from 0.08 percent to 0.60 percent. Only 5 samples were below 0.15

[5] This section includes some data published by Inouye, Forer, Reische, and Miller: *Proceedings of the Society for Experimental Biology and Medicine*, 1927, xxv, p. 153; also by Inouye and Miller: Proceedings of the International Association for Dental Research, *Journal of Dental Research*, 1928, viii, p. 450.

percent, and 2 above 0.41 percent. The largest variation in one individual was from 0.08 percent to 0.37 percent. In general, the variations in the same individuals on different days were as great as between individuals. Fifty-three samples, collected from 33 subjects *without any mechanical or chemical stimulation of flow*, had an average mucinate content of 0.25 per cent. The "probable error" was 0.06 percent. The "unstimulated" series of salivas gave essentially the same result as the "stimulated." Individual variations were of the same order in both series of salivas.

Portions of saliva, collected under lime-juice stimulation 20 minutes after a paraffin-stimulated collection, gave a decrease in mucinate content in each of 6 cases (the decrease amounting in 4 cases to as much as half the "control" amount). This accords with the frequent observation of a "watery" saliva after acid stimulation, owing perhaps to increased parotid activity. In a series of 20 cases, cigarette smoking induced no consistent change in the mucinate content. In 20 tests in which sugar ("rock candy") was used as a stimulant, following the control, there was a consistent decrease in the mucinate content. The maximum fall (> 65 percent) was not determined, because in 4 cases the extreme dilution made the nephelometric comparison unreliable. This followed stimulation by *small* amounts of sugar, but does not contradict the common observation of an increase in the mucigenous quality of saliva after the action of *large* quantities of sugar.

As a partial check on the results, the total nitrogen content of cleared and dialyzed saliva was determined in many cases. The analytic data showed the same trend and magnitude of change under varying stimuli, and corroborated the mucin amounts as calculated from the nitrogen amounts (assuming that all of the protein present was mucinate).

VI. DISCUSSION OF ORAL CONDITIONS[6]

A 0.15 percent solution of sodium mucinate has the viscid consistence of fairly "thick" saliva. Water evaporates comparatively

[6] The data in this portion of the paper were presented by Inouye, Miller, and Gies in a report on "Further studies of the properties of mucin and their dental significance," at a meeting of the New York Section of the International Association for Dental Research, Columbia University Club, 4 West 43rd Street, New York City on March 2, 1926 (proceedings of the meeting have not been published).

slowly from such a solution. Mucinate in the oral secretions, by retarding evaporation of water, normally counteracts tendencies to unphysiological desiccation of oral membranes and dental surfaces.

Mucin and mucinate readily undergo bacterial hydrolysis, and provide nourishment for microörganisms. The mucin and mucinate in a "mucin plaque" on a tooth, by constant renewal, not only serve as an adhesive surface in which bacteria are mechanically held against the enamel, but also nourish organisms thus retained.

Mucin is, in effect, a protein acid that may occur in saliva as "free" (precipitated) mucin, or as "acid salts" of mucin (some soluble, some insoluble), or as soluble normal or "basic salts." The way in which mucin may be converted from a soluble state to a precipitated form, in saliva, is *suggested empirically* by the following progressive equations (1 and 2), in which *M* represents the main mucin radical; *H*, hydrogen atom or ion; *Na*, sodium atom or ion; and *x*, a definite though unknown multiple of *H*, the names of the designated mucin products having been assigned arbitrarily:

(1) $Na_3H_{x-3}M + 2\,HCl = NaH_{x-1}M + 2\,NaCl$

Tri-sodium mucinate	Hydrochloric acid	Mono-sodium mucinate	Sodium chloride
(Soluble)	(Representing acids in general)	(Soluble?)	(Representing by-products in general)

(2) $NaH_{x-1}M + HCl = H_xM + NaCl$

Mono-sodium mucinate	Hydrochloric acid	Mucin	Sodium chloride
(Soluble?)		(Precipitate)	

In earlier experiments in this laboratory, on the action of acid media on teeth kept in moist chambers under conditions similar to those in the mouth, Prof. Gies found that smears of precipitated purified mucin, applied daily for a short time, caused marked corrosion of smooth enamel. This extreme condition—daily renewal at a given surface of an excess of mucin instead of dissolved mucinate—is not *normally*

duplicated in the mouth. But it may be approximated pathologically, especially when minutely dispersed particles of mucin or of "acid mucinate," abundantly present in saliva or in buccal or other oral secretions, pass over dental surfaces. Thus in some forms of erosion, such mucin or mucinate or both, by reacting with basic matter in enamel, might chemically dissolve the latter. This transformation may be supposed to occur in a manner suggested by equation (3):

(3) $Ca_3(PO_4)_2 + H_xM = 2\ CaHPO_4 + CaH_{x-2}M$

Tri-basic calcium phosphate, representing enamel	Mucin	Calcium phosphate, removed from enamel	Calcium mucinate
(Insoluble)	(Insoluble)	(Soluble)	(Soluble?)

The resolution of precipitated mucin, and its conversion into soluble mucinate, is suggested in equation (4):

(4) $H_xM + 3\ NaOH = Na_3H_{x-3}M + 3\ H_2O$

Mucin	Sodium hydroxide	Tri-sodium mucinate	Water
(Insoluble)		(Soluble)	

The improved quantitative method for the determination of mucin (mucinate), described in Section IV, favors closer study of oral conditions that involve the relationships of salivary mucin.

VII. SUMMARY

Mucin and sodium mucinate were prepared from submaxillary glands by standard methods, with minor modifications. The resulting mucinate was totally soluble in water.

Mucin is said to be precipitated from its solutions by salts of lead and mercury, but in the biological range of concentration this does not occur.

The reaction of maximum precipitation ("isoelectric zone") of mucin by dilute acid is pH 2.95 to 2.75. Maximum precipitation can be accurately obtained, for practical analytic purposes, by titration with $N/50$ hydrochloric acid, added drop by drop until a drop fails to increase the turbidity.

Various methods for the quantitative determination of mucin were tried. The nature of the turbidity produced by acid precipitation, while unsuited for direct gravimetric determination after separation of the mucin, suggested nephelometry.

A described nephelometric method includes preliminary clearing of the normally turbid saliva with alkali and removal, by dialysis, of salts that interfere with precipitation. By this method added mucin can be recovered from saliva with an error of less than 4 percent.

Fifty-three analyses of "unstimulated" saliva from human subjects gave an average mucinate content of 0.25 $\pm$ 0.06 percent. A series of analyses were also made on saliva collected after various types of stimulation.

Oral relationships of the data are indicated.

In conclusion the author wishes to express his sincere thanks to Prof. William J. Gies, who initiated the work and offered valuable suggestions, and to Prof. Edgar G. Miller, Jr. for his constant and generous coöperation in the research. He also gratefully acknowledges the assistance of Jessica P. Leland and Arthur B. Roth in the determinations of the mucinate content of "unstimulated" saliva.

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ADDITIONAL REMARKS ON A REFERENCE TO THE CARNEGIE FOUNDATION'S STUDY OF DENTAL EDUCATION

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On pages 595, 608, and 745 of the preceding volume of this *Journal*, the present writer referred to a prospective discussion of current conditions in dentistry, including a "proposed substitution of the term 'stomatology' for 'dentistry,' and also the two-three-graduate plan." There appears to be little occasion to follow this intention except briefly to "complete the record." Several quotations and a table will suffice.

I. STATUS OF DENTISTRY[1]

"Analysis of the situation"

"(C) Our general analysis of this situation . . . . may be summed up briefly, as follows (i-t):

"(i) Dentistry is one of the *natural* divisions of health service.

"(j) In each of the states of the United States, and in each province of Canada, dentistry is now recognized by statute and regulated by law as a service maintained primarily for the welfare of the individual patient and of the collective public.

"(k) Dentistry should continue to be organized and conducted for the benefit primarily of the patient and the public.

"(l) There is no evidence to support the theory that dentistry would be practised most effectually in the interest of the patient and the public, if portions of it were restricted to statutory medical practice. Those who have little real interest in the promotion of a cause or of a service cannot be expected to give it the most sympathetic and

[1] Quotation from a report presented about a year ago to an international dental organization, by a committee of five members of which the writer was chairman.

desirable advancement. Wherever dentistry has been restricted, in whole or in part, to the practice of conventional medicine, dental practice has failed to advance to the degree attained in the United States and Canada under the guidance of a profession separately organized to further it.

"(m) The suggestion that dental practice be so organized that 'trained mechanics' would perform the mechanical phases, working under the direction of 'oral specialists' having the M.D. degree, and on their prescriptions, appears to your Committee to offer nothing better than the prospect of general deterioration of dental practice.

"(n) It is assumed by some dentists that misjudgment or mishaps in routine dental practice are now common, but that these would not occur if dentistry were conducted or directed by 'oral specialists' having the M.D. degree. But this fantasy is not shared by sincere and experienced physicians, each of whom is well aware of the inherent and unavoidable deficiencies now existing in medical practice. ***Much research is needed for the future perfection of the practice of each profession.*** Research is the common soil from which all kinds of health service grow. The obvious need for research in oral health-service would not disappear if any part of dentistry were restricted to statutory medical practice.

"(o) The traditional inattention of organized medicine to the development of dentistry, to the prevention and treatment of the abnormalities and diseases of the teeth and oral tissues, and to the relation between oral and systemic conditions, indicates that if dentistry, or a part of it, were called 'stomatology' and included in the practice of conventional medicine, the mechanical and esthetic features of oral health-service would not attain their most desirable improvement and development.

"(p) Measures for prevention, and for the application of medical principles, are fundamental in dental practice. But the mechanical and esthetic requirements are also essential, and are becoming increasingly exacting as the corresponding intra-oral procedures multiply and attain greater complexity. The intricate methods for these difficult phases of dental practice cannot be included in the general training of physicians, *but can be developed most effectively by a separately organized profession devoted to their continual betterment.* It should not be forgotten that a dentist's knowledge of the so-called medical

sciences can, in the main, be practically applied only in proportion to his technical ability.

"(q) The unusual mechanical and esthetic demands upon dentistry have fully justified and continue to require its active development as a separately organized profession. The kind of dental service at present needed by the individual patient—in addition to advice for prevention—is now afforded by the modern trained dentist. He applies at the chair his knowledge of dentistry—of physical and biological sciences, mechanics, and esthetics—by a type of digital skill that is essential also in the many arts and crafts in engineering and which requires a bio-mechanical sense for its exercise. It is likely that the need for the present type of dental service will continue indefinitely, for it is improbable that progress in prevention will cause dental disorders requiring corrective mechanical treatment to disappear any sooner than systemic diseases that necessitate dietetic or therapeutic treatment.

"(r) Dentistry, in the quality and efficiency of its *service* to the patient, should be made the full *equivalent* of an oral specialty of conventional medicine. This can be accomplished by intimate co-operation among dentists and physicians in private practice, in hospitals and dispensaries, in health boards, etc.; and also by research and instruction in dental schools closely co-ordinated with medical schools, hospitals, and dispensaries, but without requiring the prospective general practitioner of dentistry to obtain the M.D. degree.

"(s) When dentistry, continuing its separate organization and its own system of training and practice, becomes the full *service equivalent* of an oral specialty of conventional medicine, neither the patient nor the public will have any interest in the technical question whether dentistry should be *formally* converted into a specialty of medical practice. Meanwhile, dentistry in North America cannot profit from any new relationship, which, by forced requirements or artificial character, would increase the difficulties of dental practice without assuring higher quality or greater excellence in the practitioner. Instead, dentistry, on the basis of its present separate organization, requires abundant opportunity, encouragement, and assistance fully to attain its normal stature and capabilities as a division of health service. Present dental leadership is clearly bringing about this very desirable result.

"(t) The very few practitioners who wish, and are able, to combine the responsibilities and procedures of medical practice and dental practice are now entirely free to acquire full training for the M.D. and D.D.S. degrees and to pass license examinations for admission to each statutory type of practice. If, when, and as urgent public need for this type of practitioner increases, the emergency would probably be met by individual initiative aided by medical and dental curricula co-ordinated, in at least one university, for that purpose. Such practitioners would be analogous to the few physicians who, besides holding the M.D. degree, also obtain the Ph.D. degree in, say, bacteriology. In passing, we call attention to the fact that it is not necessary for bacteriologists, before becoming experts in teaching and research in bacteriology *in medical schools*, to obtain the M.D. degree. *Most of the teachers of 'medical sciences' in medical schools have been specially trained for their work, and do not have the M.D. degree.*

"Supplementary comment"

"(D) The pertinence of the foregoing analysis may be more evident from supplementary comment regarding several matters on which our remarks have been brief or seemingly casual (u-z).

"(u) It should be remembered that, in both the United States and Canada, dentistry is *now* by law a *separately* organized profession. A change in this status cannot be brought about anywhere in the United States without definite, local, legislative action. . . . .

"(v) If dentistry were made a part of statutory medical practice, dental practitioners under this plan would also be free to practise medicine. This predicament has been earnestly considered by some of those who favor the absorption of dentistry into statutory medical practice. To avoid increased competition among medical practitioners, and to allay the apprehension of physicians on this point, these advisers suggest that the medical faculties would restrict the prescription-*writing* 'stomatologists' to a small number, whereas the prescription-*filling* 'dentists' ['technicians'] might be multiplied to insure a maximum of competition among themselves as subordinates of the 'stomatologists'.

"(w) Returns from 80 of the 88 North American medical schools for the academic years 1926–29 indicate that, of the number of persons who applied for admission,

6,420 were admitted, 3,586 were not admitted, in 1926–27;
6,495 were admitted, 4,519 were not admitted, in 1927–28;
7,014 were admitted, 5,523 were not admitted, in 1928–29.

In general the medical schools are overcrowded. Last fall Dean Lyon, of the Medical School at the University of Minnesota, stated to the assembled freshmen in the University, that 'they ought to consider medicine an overcrowded profession . . . . the only thing that would save the profession was birth control—birth control of candidates for medical schools.' Dr. W. C. Rappleye, Secretary of the Commission on Medical Education, said, at the annual meeting of the Association of American Medical Colleges in Indianapolis last October, that 'there are no indications of any probable shortage of physicians and every indication that the numbers and proportions of well trained practitioners [of medicine] will increase. . . . . The [proportionate] increase per year of physicians is larger than the increase in population.' Under these striking conditions it is obvious that, if dentistry were made a part of statutory medical practice, very few prospective dental students could be admitted to medical schools, unless the number of medical schools were greatly increased. It is equally evident that the number of prospective 'stomatologists' in any existing medical school at one time, even under the most favorable conditions, would be very small. Yet there are those who would 'improve' dental service by these means—and in an era when the existing dental practitioners should be not only better distributed but also steadily increased. How or where the prescription-*filling* dentists, or dental mechanics, or oral technicians (or whatever they might be called), are to be trained, does not seem to be included among the concerns of those who believe that dentistry may be practised satisfactorily by a few 'stomatologists' and the many assistants they would supervise, although some have suggested that the existing dental schools would 'do' for this 'minor' purpose.

"(x) It might be thought by some that a formal allusion to the present reality of dentistry's separate organization as a profession, and to the public desirability of *continuing* that separate organization, would indicate or imply hostility to or discord with other divisions of health service. Your Committee feels, however, that a self-respecting assertion of the individuality of dentistry would indicate devotion to

dentistry, promise achievement in its behalf, and promote public recognition of the importance of dental practice, but could not cause ill will or opposition in any other group of health-service practitioners. Every member of the Committee, and we believe every member of . . . . [this organization], hopes that co-operation and sympathy between dentists and physicians, and between dentists and other kinds of health-service practitioners, will continue to grow stronger, and gladly accepts every opportunity to foster good feeling and understanding. The steady improvement in dental education, including more effectual application of the medico-dental sciences and of oral medicine—and the growing interest in clinical dentistry at medical schools—are furthering these desirable developments.

"(y) Separate organization does not necessarily imply hostility to anything. It appears entirely unwarranted for any one to assume that *continuance* of the separate organization of dentistry will cause discord, disharmony, or anything like it, between, say, dentists and physicians. It is often said that the dental and medical professions are steadily drawing closer together. All in . . . . [this organization] are happy to note the increasing harmony, but we see also that the *separate* organization of each profession *has not* prevented this desirable growth of accord, which is undoubtedly based on increasing mutual understanding and respect. It is unreasonable to assume that *continuance* of separate organization would prevent closer agreement and co-operation between the two groups of practitioners. If separate education and organization necessarily expressed hostility or discord, one wonders why schools or departments of a university are separately organized, and why, for instance, the anatomists, bacteriologists, and physiologists in medical schools have been separately trained in their sciences and also separately organized into national societies? The answers to all such questions are the same: each is separately organized for the promotion of interests and causes that would not be so well advanced if they were in the care of persons less attentive to them and having more intimate concerns. This is obviously the essential purpose and condition of all constructive organization—to give *intensive attention to interests and causes that otherwise would be minimized or neglected*? The International Association for Dental Research has just closed a two-day meeting at the Northwestern University Dental School, which, in the unanimous

opinion of the members and guests, has been an extraordinary success. No organization *in any other profession* would be willing to devote two whole days exclusively to a discussion of the results of *dental* research. By separate organization of the active workers in dental research, public interests that otherwise would be minimized are given the attention they deserve—and no other cause is or can be thereby injured or adversely affected."

II. PROPOSED SUBSTITUTION OF THE TERM "STOMATOLOGY" FOR "DENTISTRY"[3]

"(z) The assumption that dentistry would be something more or better than it is, if all or a part of it were called 'stomatology,' exemplifies a readiness to attach more importance to labels than to contents, or more significance to make-believe than to reality. The term 'medicine' originally meant to remedy or cure. ['*Medicina* (ars)'—healing art; '*medicina* (res)'—a remedy; '*medicus*'—of healing; also a physician.] Today 'medicine,' by including measures to *prevent* sickness and by throwing *etymology* overboard, means not only to remedy or cure, but also *quite the contrary*—to make remedial or curative treatment unnecessary. As medical knowledge has advanced and medical procedures have been changed in consonance, including the development of prevention and of surgery, new meanings have been imparted to the term 'medicine.' But instead of replacing 'medicine' by a succession of words intended to indicate *etymologically* the broader or deeper modern meanings, the import of the original term has been conveniently enlarged as the scope of medical practice has expanded. Thus, the term 'medicine' has come to mean more and more as *physicians*, without restricting themselves to its *etymology*, have included *more and more in its significance*. Along the same line of reasoning one sees clearly that, although *etymologically* the term 'dentistry' refers only to the teeth, nevertheless logically it also comprehends the contiguous parts and their systemic relationships. Certainly by *usage* in conformity with this logic, and also with the criteria of common sense, the term 'dentistry' has *acquired* the broader meaning suggested by the more detailed and lengthy term 'oral health-service.' By logic, usage, and convenience, the term 'dentistry' has thus become the *practical* equivalent of 'stomatology,' which

[3] Footnote 1 applies to this quotation also.

etymologically means the sum of scientific knowledge concerning the mouth, but *etymologically does not include the rest of the body* (although 'stomatology' is now *used* by some to include systemic relationships with the mouth). This being true, we are unable to agree that 'dentistry' and 'dentists,' which are terms that have long represented the earnest and aspiring endeavors of generations of faithful practitioners of oral health-service, should be formally replaced for *etymological* reasons by 'stomatology' and 'stomatologists.'"

III. TWO-THREE-GRADUATE PLAN

The two-three-graduate plan has recently been fully outlined in this *Journal*.[3] Three quotations will conveniently supplement that outline.

"The plan, a triparite program of essentials, is like a skeleton rather than a fully organized body, and therefore, in its use, is *susceptible of wide variation and of free expression of preferences*. Its primary phases can be adopted separately, experimentally, and gradually, without impairment of the system into which they may be fitted. *It would impart both elasticity and diversity to dental education, for it is neither a mould nor a straitjacket*. It suggests conditions that are suitable for general adoption as *minima*. By lengthening the professional years so that three would have the content of four, schools that now prefer a conventional two-four plan without reference to graduate work could save a year of the practitioner's time for professional service. Tuition fees, teaching salaries, etc., would presumably be readjusted accordingly. The *optional graduate phase provides in effect, by addition, two-four, two-five, and two-six plans, culminating in all aspects of oral specialization and research*. The plan presents a basis for close coöperation between medical and dental schools, and favors an evolution in the relationship between medicine and dentistry that would be *entirely natural and in full accord with the future responsibilities of each*." (Italic not in the original.)[4]

"The educational plan that seems to promise the widest usefulness in the United States is what we have conveniently called the 'two-

[3] Gies: Some of the findings and conclusions of the Carnegie Foundation's study of dental education: the two-three-graduate plan. *Journal of Dental Research*, 1927, vii, pp. 441–456.

[4] Gies: Carnegie Foundation's Bulletin on Dental Education, No. 19; 1926, p. 204.

three-graduate plan.' This plan is conservative in its demand for time; progressive in its elevation of spirit, purpose, and means, yet moderate in its economic exactions and in its deviation from prevailing views; and also *elastic in its relation to the preferences of individual schools.* . . . . Other plans, such as the 'nought-five,' the 'one-four,' or *the two-four form* of the two-three phase of our plan, *have much to commend them.* . . . . I venture to predict that as soon as the two-three-graduate plan is fully understood, especially the significance of its academic and *graduate* phases, and the fact that *three long professional years will be more useful than four short ones* where the latter are now preferred—then that plan will become the accepted *basis* for a stabilized procedure in dental education. The plan will grow in favor, after partisanships die out, *because it carries the foundations for an enduring program in professional education in general.*" (Italic in last sentence not in the original.)[5]

"From other points of view it *may be questioned whether it is desirable to require for all forms of current medical practice the detailed and diffuse information of the present overloaded undergraduate medical curriculum.* The development of optometry, for example, plainly expresses a realization, among well-informed laymen particularly, of the lack of proper coördination between the training of the general practitioner of ophthalmology and the service he commonly renders. Dentistry, by grounding its health service on the medical sciences, and by aiming to correlate the instruction in these sciences with the obvious needs of the beginner in dental practice, *has set an example of very desirable adaptation of means to ends. It can assuredly attain its highest serviceability by continuing along this path of growth.* There is nothing of consequence for dental practice in a curriculum for the M.D. degree that cannot suitably be included in the dental curriculum, or which cannot soon be learned by the young dental graduate as he teaches himself and grows in capacity from experience. There is no good reason to assume that anything important that may not be taught to a candidate for the M.D. degree, or any other degree, cannot or will not be learned by him shortly after his graduation; and very much that may be taught to a prospective physician can be learned by a dentist outside of the medical school. These self-evident truths are often overlooked *by those who would mold all types*

[5] Gies: *Journal of Dental Research*, 1927, vii, p. 453.

of health-service education into the straightjacket of the undergraduate medical curriculum." (Italic not in the original.)[6]

The accompanying table indicates, in a general way, the growth of interest in the various plans now being tested in the dental schools in the United States.

CLASSIFICATION OF THE UNITED STATES DENTAL SCHOOLS ACCORDING TO CURRICULA

Minimum academic requirement for admission to first dental year

YEAR	PLANS[1]							NUMBER OF SCHOOLS	
	0–4	1–4[2]	0–5	2–3-graduate (professional curriculum in three years)		2–4	3–4	Total	Require at least two academic college years for admission
				Conventional length	Including summer sessions ("quarters")				
1923–24	22	21	0	0	0	0	0	43	0
1924–25	20	21	0	0	0	2	0	43	2
1925–26	15	25	0	1	0	2	1[3]	44	4
1926–27	2	25	6	2	0	4	1[3]	40[4]	7
1927–28	1	23	3	4	0	8	1[3]	40[4]	13
1928–29	0	25	0	5	0	9	1[3]	40[4]	15
1929–30	0	23	0	5	2[5]	8	0	38[6]	15
Present group totals	23			7[5]		8		38[6]	15

[1] The first numeral in each heading-pair indicates years of work in an academic college; the second, years in the undergraduate professional curriculum.

[2] Practically all of the 1–4 schools admit qualified students to advanced standing on a 2–3 plan.

[3] Rochester: opened in 1925; has had no dental students; in dentistry now offers graduate work only.

[4] Ohio (Cincinnati) and Vanderbilt—1–4 schools—were discontinued in 1926. Beginning in 1926, no new students were admitted to Tulane and Cincinnati (proprietary), both 1–4 schools. They were discontinued in 1928 and 1929, respectively. These four schools are not included in this total.

[5] At Northwestern, where several alternative plans are open to election but one academic-college year is the minimum admission requirement, the four-year curriculum may be completed in three years of twelve consecutive quarters (including summer quarters).

[6] Beginning in 1929–30, Rochester (3–4) became a graduate school in matters relating to dentistry. Denver (1–4), about to discontinue, has not admitted new students. These schools are not included in this total.

[6] Gies: Kent's Higher Education in America; Chapter V, the School of Dentistry, p. 128. (In press; the quoted paragraph was in the original manuscript as submitted in August 1927.)

A QUANTITATIVE EXAMINATION OF THE MOUTH FLORA UNDER DIFFERENT DIETARY CONDITIONS[1]

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I. INTRODUCTION

The first and apparently the only work reported in the literature dealing with a quantitative examination of the mouth flora is that of Kligler and Gies (1915), who made an excellent study of the amounts and species of bacteria from carious teeth and from teeth in normal mouths. They did not, however, follow any case over an extended period of time, nor make any reference to the influence of different diets upon the mouth organisms. Since no similar quantitative estimation

[1] Presented at a session of the Sixth General Meeting of the International Association for Dental Research, Washington, D. C., March 25–28, 1928. See abstract: *Journal of Dental Research*, 1928, viii, p. 435.

has been reported for the bacteria of pyorrhetic conditions, the following work deals with an attempt to apply the methods of Kligler and Gies to a study of the mouth flora of men showing different stages of the inflammatory type of pyorrhea alveolaris (parodontitis—Simonton 1927). This type of pyorrhea seemed more probably connected with microbial infection than did the other or non-inflammatory type (paradontosis) in which the essential lesion involves the bone. Examinations were also made of a series of parodontic cases selected for metabolism and clinical study of alveolar atrophy by Dr. Martha R. Jones and associates.

It was thought important to learn whether dietary factors have any influence upon relative or actual numbers and kinds of mouth flora; that is, to determine whether a man on a high carbohydrate diet might have a type of flora different from that of one on a high protein diet, and whether, by reversing the type of diet, the type of flora might be reversed. The influence of certain organisms of the Lactobacillus group upon the intestinal flora of man leads one to surmise that such changes might take place in the mouth. The work of Löhr (1926) also follows the same general idea, in that he believes it possible to "alter the character of the normal bacterial flora in the human organism by altering the medium." He found that the degree of acidity in the stomach and in the upper gastro-intestinal tract is a determining factor in the growth of different bacteria. If such changes in the microbiology occur in the mouth, there is this question to answer: Are oral bacteria more influenced by endogenous conditions owing to effects of dietary changes upon the general systemic body reactions, or by exogenous conditions due to the kind of pabulum in the mouth that might serve as a culture medium for the organisms.

II. SOURCE OF MATERIAL

Men in the state prison at San Quentin, California, were used in this work because their dietary conditions could be easily controlled and because material could be obtained from them over an extended period of time. A group of seven men was chosen; three of them with normal mouths, two with medium pyorrhea, and two with advanced pyorrhea of the inflammatory type. One from each group was placed on a high carbohydrate diet, and one from each on a high protein diet.

After a time they were all changed to the opposite diet, the protein to the carbohydrate and vice versa. The seventh man, who had a normal mouth condition, was given a combination of the two diets. Men with long prison terms were chosen, but one or two secured parole before many examinations were made. As cases of advanced inflammatory type within the required age limits were difficult to obtain, several months elapsed before the desired men could be found to fulfil satisfactorily all requirements. For this reason not all of the men started the diets at the same time, and they did not all have an equal number of examinations. The normal cases and those with medium pyorrhea were under observation for the longest periods.

The subjects, all between the ages of 25 and 35, were physically sound (no drug addicts nor men with positive Wassermanns were taken), and were apparently willing and anxious to coöperate in regard to the diets. Complete x-ray pictures were taken of each mouth and a dental examination was made by Dr. F. V. Simonton, who recorded the degree of detachment, the pocket formation, presence of caries, loss of teeth, and other details. Besides this group of men there was also a another (Group II), composed of ten men having pyorrhea with varying degrees of bone resorption. These men had been used in metabolism tests by Dr. Martha R. Jones, and for a number of months had been on protein and carbohydrate diets prescribed by her. Six of them were on the carbohydrate diet, four on the protein diet. Material for bacteriological examination was also obtained from these men, but not so regularly nor so often as from the first group. Toward the latter part of the work, seven of these men had their diets reversed for a short time. The work with this group, however, was not carried on so systematically as with Group I.

III. CLASSIFICATION OF THE MEN BY ORAL CONDITIONS

The men in Group I were classed into three groups according to oral conditions, degree of detachment at the gingival margins having been used as the standard of comparison (Fleming, 1926). Nos. 1, 4 and 8 had only a slight gingival detachment and were therefore considered as normal controls, although their mouths were by no means perfect. Nos. 2, 5 and 6 showed a moderate degree of detachment and of inflammation, and were considered as having medium pyorrhea.

Nos. 3 and 7 were considered excellent examples of advanced inflammatory pyorrhea. The latter had many deep pus pockets, fetid odor, and much stagnant debris within the pockets, together with much calculus formation.

Among the second group of men were several whose mouths not only showed marked bone resorption, but had deep pockets and also a certain amount of inflammation. For comparative purposes, however, they were grouped according to the method already mentioned. Nos. 11, 13 and 16 showed very little recession and were placed in the normal group. Nos. 9, 10, 15, 17 and 18 were considered to have incipient or medium pyorrhea. Nos. 12 and 14 fell into the advanced pyorrhea group.

IV. DIETS

The different men in the two squads were given meals, according to a schedule previously arranged by Dr. Martha R. Jones, of such caloric values that the protein diet produced a decided acid reaction in the body, while the carbohydrate induced a systemic reaction that was more toward the neutral point. Neither diet was, strictly speaking, pure protein nor pure carbohydrate, but each was so balanced toward one side or the other that the urinary pH could be relied upon to indicate whether the men were adhering to the rules. Weekly pH determinations were usually made on composite samples of urine. The pH values for the protein squad averaged about 5.7 with extremes of 5.2 and 5.9, while those for the carbohydrate squad averaged about 6.4 with extremes of 6.2 and 6.9. In this way the two groups could be roughly differentiated, those with urinary pH values above 6.2 belonging to the carbohydrate squad; below 5.9, to the protein. If a sudden change in the urinary pH was found, violation of the dietary rules was generally suspected.

The man on the balanced diet (No. 8) ate his meals alternately with the two squads, having breakfast, for example, with the protein men, lunch with the carbohydrate men, dinner again with the protein squad, and so on. He was free to choose and eat as much as he wished from either table, so that his choice would be representative of the average American fare. His choice was largely on the carbohydrate side, the average urinary pH value being 6.4.

Tables 1a and *1b* illustrate the types of diet used for each group at the daily meals. They are both copies of the weekly fare of one man, No. 15—*Table 1a* gives his weekly fare on the protein diet; *Table 1b*, after the change to the carbohydrate diet. It is interesting to note that

TABLE 1 a

Type of protein diet for week ending 5–21–27

CASE NO.	AVERAGE pH OF URINE	PORTIONS							KIND OF FOOD	UNIT QUANTITY
		S	M	T	W	T	F	S	1. Breakfast	
15	pH 5.2	2	3	2	2	2	2	0	Water	1 cup
		1	1	1	1	1	1	1	Eggs	2 eggs
		1	1	1	1	1	1	1	Bacon	2 slices
		1	1	1	1	1	1	1	Bread	1 slice
		1	1	1	1	1	1	1	Coffee	1 cup
		1	1	1	1	1	1	1	Gravy	1 tablespoon
									2. Lunch	
			1			1	1	1	Stew	1 ladle
		1				1			Veg. salad	3 tablespoons
		1		1	2				Meat, roast	1 slice
			0			1			Tomatoes	3 tablespoons
		1			1				Peas	3 "
				1				1	Macaroni, cheese	3 "
		1	1	1	1	1	1	1	Butter	1 pat
		1	1	1	1	1	1	1	Bread	1 slice
		1	1	1	1	1	1	1	Coffee	1 cup
									3. Dinner	
				1	1				Meat, roast	1 slice
			1			1	1	1	Meat, fried	1 "
			0		1	2			Tomatoes	3 tablespoons
								1	Corn	3 "
					1				Peas	3 "
				½					Macaroni, cheese	3 "
			1	1	1	1	1	1	Butter	1 pat
			1	1	1	1	1	1	Bread	1 slice
			1	1	1	1	1	1	Coffee	1 cup

the urinary pH in the first table is 5.2— in the second, 6.9— showing that the urine was affected by the dietary change. Both squads were given a stew which, for the first four months of the six, contained more meat and no potatoes for the protein men and less meat with potatoes

for the others, together with about the same type of vegetables for both. For the last two months, however, the meat was removed

TABLE 1 b

Type of carbohydrate diet for week ending 6–4–27

CASE NO.	AVERAGE pH OF URINE	PORTIONS							KIND OF FOOD	UNIT QUANTITY
		S	M	T	W	T	F	S	1. Breakfast	
15	pH 6.9	3	3	3	2	2	0	2	Water	1 cup
			1		1			1	Rolled oats	1 ladle
		1				1			Corn-meal mush	1 "
				1			1		Wheat cakes	2 cakes
		1	1	0			0		Butter	1 pat
		1	1	0	0	0	0		Bread	1 slice
				1½					Syrup	2 tablespoons
		1	1	1	1	1	1		Coffee	1 cup
		5	4	1	4	1	1		Sugar	1 teaspoon
		2	1	2	1		1		Milk	½ cup
									2. Lunch	
		1	1	1½	1	1½	1	2	Stew	1 ladle
		2	1	1	2	2	1½	2	Bread	1 slice
					1		0	2	Bread pudding	1 dish
						1			Chocolate pudding	1 "
		1	1	1	1	1	1	1	Coffee	1 cup
		1½	2	1	1	1	1	1	Sugar	1 teaspoon
		1	1	0	1	1	1	1	Butter	1 pat
									3. Dinner	
				1					Fried potatoes	3 tablespoons
					1		1		Baked "	1 med. size
						1		1	Browned "	1 " "
					1		2		Corn	3 tablespoons
							1	1	Onions	3 "
		1	1						Lettuce	3 "
				⅔		1			Spinach	3 "
						0	½		Macaroni	3 "
		0	1	1	1		1		Apple pie	1 piece
					1	1			Lemon pie	1 "
		1	1	1	1	1	1	1	Coffee	1 cup
				1	1		1	1	Sugar	1 teaspoon
		1	1						Cake	1 piece
				1	1	1	2	1	Bread	1 slice
				1	1	1	1	1	Butter	1 pat
		5	3	4					Chocolate creams	

from the stew given to the carbohydrate men and no sugar was allowed in the coffee of the protein men, in an attempt to accentuate the effects of both diets. A weekly allowance of candy was regularly given the carbohydrate squad during the whole period.

As a rule the men adhered quite strictly to these fares, especially the ones who, like Nos. 3 and 7, had jobs that made it difficult for them to secure anything extra. Several of the other men, however, had work which presented temptation. No. 4 confessed to having consumed several chocolate bars when on the protein diet, so that a few irregularities in his results may be due to such a factor. In dealing with human subjects one usually encounters certain variables. In experiments like these, allowance should be made for such individual variations both as to taste and character. Offered the same food, one man might take little or nothing of one article while another might ingest a large amount of it.

V. METHODS

1. Collection of material

The material for the quantitative bacterial examination was collected by the same person (W. C. F.), in the same manner, with the same instruments, under approximately the same conditions, and from the same teeth from each man at each time, in order that a definite basis for comparisons might be maintained. Weekly collections were made whenever possible. One of us (Fleming, 1926) had previously found that the greatest degrees of gingival detachment per tooth, and therefore the deepest pyorrheal pockets, were usually around the central incisors and the first molars. Material was therefore taken from the central incisors and the first molars, when possible, on each side and on both upper and lower jaws, making a total of eight teeth in each man. Where any of these teeth were missing, the adjacent molar or incisor was used. Material was scraped from around the gingival margins of all four tooth surfaces and from the depths of any pockets, and carefully rubbed off onto a sterile, bent, glass rod. This rod was held in a cotton-and-gauze stopper fitting into a glass vial 2.5 inches high by 1 inch in diameter, containing small glass beads. The whole container was sterilized by dry heat, and just before using was weighed

accurately to 0.1 mg. The weight was obtained again immediately after the collection, and the amount of material in milligrams recorded for each man. As much care as possible was used in obtaining the scrapings in order to avoid any chance air contaminants. Apparently the method was reliable, since obvious contaminations rarely appeared in the cultures.

Cultures were made as soon as possible after the debris was obtained from the required men, usually within two hours. The inoculated plates were then returned to the laboratory in San Francisco, where some of them were made anaerobic according to the method described by Boez (1927). All of the cultures were placed in an incubator at 37°C.

2. Dilutions for inoculation

After obtaining and weighing the material, 10 cc. of sterile physiological saline were added to each vial, which was well shaken to obtain a uniform suspension. One cc. of this 1–10 dilution was then placed into 9 cc. of saline in the same type of sterile vial, making a 1–100 dilution. Dilutions were made in this manner up to 1–100,000 or 1–1,000,000, when necessary. In making the inoculations judgment had to be exercised in selecting the dilution. It was found that one could judge quite accurately from the weight of debris; the heavier inoculum for the lighter weights, and vice versa. A definite amount of the desired dilution was pipetted into each plate, the medium was added and thoroughly rotated to secure an even distribution of colonies.

3. Media

(*a*). *Aerobic blood-agar plates.* Veal-infusion agar was used as a base for all the different plates, modified for each type of medium desired.

The blood-agar plates were poured from tubes of plain veal-infusion agar (pH 7.4) having 1 cc. of sterile defibrinated sheep-blood added just before use (Brown, 1919). These plates were incubated aerobically at 37°C. for 48 hours, and then removed to room temperature over night before counting the colonies. By always allowing this length of time, well defined and characteristic colonies of streptococci, mostly of the alpha type, were present and could be readily differentiated.

One colony of each morphologically different streptococcus was picked under a dissecting microscope and transferred to a slant of glucose veal-infusion blood agar. Colonies of gram-negative diplococci and of small gram-positive rods were also isolated from plates belonging to each case, though not so regularly as were the streptococci. After 24 to 48 hours of incubation, the cultures were planted into plain beef-infusion broth (pH 7.5) and later inoculated by means of a pipette into different carbohydrate media. The yeast-broth medium of Ayers and Rupp (1920), plus 1 percent Andrade's indicator, was used as a base for the "sugars."

The combination of the Brown-Holman classification for streptococci was used, according to the tables in the University of California Medical Bacteriology Syllabus, p. 150 (Meyer, Anderson, et al., 1925). The gram-negative diplococci and the small gram-positive rods were also identified by cultivation in the yeast carbohydrate media. The tables in the same syllabus were used for their classification.

(*b*). ***Anaerobic ascitic-fluid agar plates.*** Poured plates of veal-infusion agar (pH 7.4), to which was added 1 cc. of sterile ascitic fluid just before using, were incubated anaerobically in a tall museum jar. The latter was made anaerobic according to the method of Boez (1927), in which the catalytic agent is outside the jar. The plates were incubated for a week at 37°C., to give plenty of time for the development of such slow-growing organisms as *Leptotrichia buccalis*. There seemed to be sufficient moisture to prevent any drying of the media during this period, and viable organisms could always be isolated from the plates. Subcultures were made from characteristic colonies of *Micrococcus gasogenes*, *Leptotrichia buccalis*, fusiform bacilli, and short gram-positive rods into tubes of deep brain medium, and of the streptococci on to glucose blood agar slants. The latter were usually incubated aerobically. No strictly anaerobic streptococcus was ever found, nor any sporulating anaerobes.

(*c*). ***Anaerobic glucose veal-infusion agar plates*** (*pH 4.8–5.0*). To determine what form of organisms would grow in a very highly acidified medium, poured plates were made, using glucose veal-infusion agar with a pH of 4.8 to 5.0. These were incubated under anaerobic conditions. Streptococci and gram-positive aciduric rods, with occasionally a few gram-positive thread forms, were the usual types isolated from

this medium. The rods were transplanted on to either acid agar-slants or to glucose blood agar-slants, and were usually incubated aerobically. The thread forms were put into brain medium and the streptococci onto glucose blood agar-slants. The yeast sugar medium was used for the differentiation of both the streptococci and the rods. Brom-cresol-purple milk was also used for the latter.

(*d*). *Media for sporulating anaerobes.* To determine the presence of sporulating anaerobes, several cc. of 1–10 dilution from each specimen were heated to 80°C. for thirty minutes. One cc. of this material was inoculated into heated brain medium and 1 cc. into a poured glucose agar-plate. Both were incubated anaerobically at 37°C. for a week. No sporulating anaerobes ever developed, only an occasional spore-forming aerobe of the subtilis group.

4. Counting methods

Two plates of each type of medium were poured for each specimen; 0.1 cc. of dilution 1–10,000 and of 1–100,000, respectively, were usually taken for the aerobic blood agar-plates; 1 cc. each of dilution 1–10,000 and 1–100,000 for the anaerobic ascitic-fluid agar-plates, and 1 cc. of either dilution 1–10 and 1–100, or of 1–100 and 1–1000, for the acid agar-plates, depending on the amount of material obtained. These dilutions were the most satisfactory for counting purposes, but, of course, this means that the method is quite selective. Only those organisms were grown which, both the most viable and the most numerous, remained in the higher dilutions. For this reason, organisms seen in the direct smears of the undiluted material might be found in only the lower dilutions of 1–10 or 1–100. But since these few colonies would be masked by myriads of other more rapidly growing organisms, it would not be satisfactory to use these dilutions in media of the usual H-ion concentrations. The method, therefore, is useful only for the more outstanding and easily growing bacteria.

The total number of colonies on each plate was counted under a dissecting microscope and the average taken for each kind of medium. The average number for the total material was then calculated and divided by the weight in milligrams, thus obtaining the number of organisms per milligram for each condition used. After obtaining the total number per plate, the average percentage of each type of colony was estimated.

Since no quantitative estimation of spirochetes could be obtained by the methods used, it was hoped that some idea of their relative numbers might be found from a smear count. Accordingly, 0.1 cc. of 1–10 dilution from each specimen was spread evenly on a slide, stained by Gram's method (carbol-fuchsin counterstain), and all the organisms counted in twenty fields. From the relative numbers of spirochetes, the average percentage per twenty fields could be calculated.

VI. QUANTITATIVE FINDINGS

After repeated examinations of material from both groups of men over a period of about six months, the work at the prison was discontinued. The results are expressed graphically by means of tables and charts. *Tables 2* and *3* give detailed protocols of the work on the men in Group I, and *Table 4* on those in Group II, for both squads (protein and carbohydrate). They show the pH of saliva on each day of examination, the amount of material in milligrams collected each time, and the results for each type of culture medium used—aerobic blood agar-plates, anaerobic ascitic-fluid agar-plates, anaerobic acid agar-plates (pH 4.8–5.0), and anaerobic glucose agar-plates inoculated with heated material. Under the column for each medium is indicated the average number of organisms per milligram, and the average percentage of the different kinds of organisms, for each condition. The average number of spirochetes per twenty fields on a stained slide is also shown for each examination.

Each man in Group I was examined at least once, in most cases twice, before going on the different diets. The numbers of the examinations when the diets were begun, and when they were changed, are indicated by different symbols. None of the men in the second group were previously examined, because they had already commenced their diets before this work was begun. However, if any changes were made, they are also noted. Nos. 1, 2, and 3 of Group I were originally with the carbohydrate squad; later were changed to the protein. Nos. 4, 5, 6, and 7 began with the protein squad; subsequently were transferred to the carbohydrate. No. 8 ate a balanced diet throughout, alternating his daily meals with each squad. In Group II, Nos. 9, 10, 11, 12, 13, and 14 began with the carbohydrate squad; Nos. 15, 16, 17, and 18 with the protein. Not all of this second group had the diets reversed.

TABLE 2: First part. Group I

BLOOD AGAR-PLATES (AEROBIC)

Case no. and age	No. exam.	Degree of detachment in mgm.	pH of saliva	Amount of debris in mgm.	Average number of organisms per mgm.	Average per cent streptococci			Average per cent plate incidence							Streptococci								
						Alpha	Beta	Gamma	Gram − diplococci	Small gram + rods	Sarcinae	Staph.	Gram + filaments	Mic. tetrag.	Large gram + cocci	S. saliv. 2.1	S. saliv. 2.2	S. mitis 1.1	S. mitis 1.2	Inulin +	S. equinus	S. ignavus	S. equi	S. pyogenes 1.1
No. 1	I*		7.3	34.7	2,800	100										+								
Age 32	II		7.9	8.8	1,293,000	100										+			+					
Normal	III†	0.7	7.9	39.8	748,000	26.8		15.8	58.1									+						
mouth	IV		7.4	15.4	3,330,000	89.5			2.0	8.5						+								
	V		7.8	46.4	960,000	59.5				40.5								+ +						
	VI		7.6	12.7	401,000	73.5			26.4									+ +	+					
	VII		7.8	21.9	438,000	77.4			6.0	16.6											+			
	VIII		7.6	16.8	369,000	97.3			2.7								+	+ +						
	IX		7.5	25.4	370,000	52.0			35.7	12.2						+		+ +	+					
	X		7.6	16.0	493,000	47.2	1.0		21.4	30.2						+		+ +	+ +				+	
	XI		7.5	19.2	776,000	59.0		3.3	11.2	23.0								+ +	+ +			+		
	XII‡		6.5	11.4	138,000	63.6		26.6	6.0	3.0								+ +	+ +					
	XIII		7.9	27.4	398,000	64.3	1.8	7.1	24.8			0.35						+	+ +					
	XIV		7.9	18.4	464,000	66.8			25.1	8.1									+ +					
	XV		7.7	26.5	828,000	83.6			5.2	11.1						+			+ +					
	XVI		7.5	26.8	832,000	78.6			9.5	9.2			1.2			+		+						
	XVII		7.8	17.8	542,000	26.4			21.6	35.2					16.7	+		+		+				
No. 2	I*		7.1	53.1	14,100	40						40		20			+							
Age 28	II	1.7	7.8	66.3																				
Medium	III†		7.2	81.8	684,000	54.2	2.08			43.7								+						
pyorrhea	IV		7.5	83.8	237,000	83.8			0.55	15.6									+					
	V		7.4	64.0	164,000	93.6			2.2			3.1										+		
	VI		7.2	40.8	408,000	86.9	1.0	1.7	2.3	7.1						+	+		+					
	VII		7.2	50.0	108,000	77.7		1.8	1.8	13.5		4.9				+		+ +	+					
	VIII		6.9	46.8	1,100,000	71.3			8.7	11.1					8.8	+		+ +	+					

	IX		7.1	68.0	139,000	87.5	0.35	1.1	0.35	4.8					5.6			+		+				+
	X		7.2	59.0	642,000	56.4		18.1	15.3				5.1		5.1	+	+	+						
	XI‡		7.0	42.1	646,000	70.0	1.0	8.0	11.0	8.0		2.0				+	+				+			
	XII		6.7	53.0	556,000	65.5	1.0	6.2	5.1	21.1					1.0		+	+	+					
	XIII		7.2	58.9	483,000	86.7	1.5			11.7						+				+				
	XIV		7.6	45.0	237,000	90.3									2.8	+		+	+					
	XV		7.2	56.7	223,000	90.5			4.6						3.6			+	+					
	XVI		6.9	50.6	839,000	52.6				47.4							+	+						
No. 3	I*		7.4	82.0	3,170,000	73.5			18.6	7.9							+		+					
Age 35	II†		7.3	95.9	728,000	47.2			52.7							+			+					
Advanced	III		7.3	59.3	214,000	60.9		1.0	4.7	3.0					24.9		+		+					
pyorrhea	IV		7.3	54.8	1,905,000	67.5			2.7	27.9					1.8			+						
	V		7.3	86.0	1,604,000	81.5			3.8	8.4					6.1	+		+						
	VI		7.5	68.7	720,000	61.2			29.0	9.7						+								
	VII		7.4	123.0	150,000	70.3		12.5		16.8								+	+	+				
	VIII		6.8	83.4	735,000	62.7	0.8	2.0	20.9							+				+			+	
	IX		7.2	154.5	436,000	60.6		7.2	14.3						12.8	+				+				
	X		7.3	99.0	1,404,000	84.4			8.2	5.5					1.8				+			+		
	XI‡		7.3	73.4	1,092,000	68.7			22.0	8.1			1.2							+		+		
	XII		7.0	95.0	954,000	66.1			15.3	15.3						+		+						
No. 8	I*	3.3	7.1	30.7	9,700	33.3				66.6							+							
Age 24	II		7.3	22.4	500,000	95.5		2.6				1.7							+					
Normal	III§		6.1	16.0	2,370,000	84.4			15.0	0.6									+					
mouth	IV		6.9	12.3	211,000	57.8			2.5	39.5									+					
	V		7.2	41.3	811,000	27.6			0.25	72.1									+					
	VI		6.7	22.8	183,000	46.4			50.3			3.6							+					
	VII		7.2	24.7	133,000	90.6			6.2	3.1									+					
	VIII		7.2	19.3	740,000	65.1			4.4	26.3		3.8						+	+					
	IX		7.4	24.7	64,700	54.1			45.8							+								
	X		7.2	19.0	384,000	87.6		9.2		3.0						+								
	XI		7.4	5.8	443,000	62.2		15.2	3.0	0.6	6.9				11.8					+				
	XII		7.1	7.7	223,000	74.2			2.7	24.2		3.0						+		+				
	XIII		7.4	43.3	401,000	69.2			13.2	16.6		0.8								+	+			
	XIV		7.3	31.4	1,410,000	50.0			40.9	4.5									+	+	+			

* Prison diet.
† Carbohydrate diet.
‡ Protein diet.
§ Balanced diet.

TABLE 2: Second part. Group I, continued

Case no. and age	No. exam.	ASCITIC-FLUID AGAR-PLATES (ANAEROBIC)											ACID (pH 4.8–5.0) AGAR-PLATES (ANAEROBIC)					GLUCOSE AGAR-PLATES (ANAEROBIC)		
		Average number of organisms per mgm.	Average per cent plate incidence										Average number of organisms per mgm.	Average per cent plate incidence				Number of organisms per mgm.	Heated material	Stained slides; per cent of spirals
			Strep.	Lepto. buccalis	Mic. gasogenes	Large gram − diplococci	Small gram + rods	Large gram + cocci	Staph.	Gram − threads	Thin gram + threads		Strep.	Small gram + rods	Large gram + fil.	Staph.		Gram + aerobic spore rods	
No. 1	I*	4,800	82.3					17.7					97		100			0		3.2
Age 32	II	1,556,000	94.8			5.2							102		100			0		0.8
Normal	III†	1,349,000	99.0	1.0									15	32.4	67.5			0		2.9
mouth	IV	3,840,000	79.6		10.1		10.1						48	42.2	57.7			0		2.7
	V	913,000	78.4		3.0		18.4						18	100				0		1.3
	VI	734,000	100.0										23	100				0		3.6
	VII	1,333,000	94.9		3.3		1.8						54	39.5	60.5			0		0.7
	VIII	366,000	82.8			17.2							93		100			0		0.09
	IX	374,000	84.9	0.1			15.0						132		100			0		0.3
	X	322,000	73.8						15.8		10.4		16		100			0		4.3
	XI	1,220,000	72.6		13.5	7.2	1.2			5.5			1	33.3	66.6			0		2.3
	XII‡	137,000	93.0	0.4	1.6		1.9				3.1		7	50.0			50.0	0		0.4
	XIII	456,000	72.7	0.6	5.2	11.1	3.2				7.2		0.1		100			0		2.2
	XIV	735,000	76.2	0.2	3.5	4.8	10.4				4.8		1.3	100				0		2.2
	XV	1,120,000	95.5	1.0	3.5								1.8	25	75			0		2.9
	XVI	666,000	84.2		2.0	5.8	2.7				5.3		0.3	100				0		6.5
	XVII	2,210,000	90.2		4.8		2.5				2.5		8.0	54.8	45.2			0		5.5
No. 2	I*	3,800	100										3.7		100			0		12.6
Age 28	II	23,900	97.2						2.7				150		100			0		7.9
Medium	III†	566,000	96.8	1.7	0.9		0.92						227	17.7	82.2			0		1.5
pyorrhea	IV	1,870,000	34.8	0.88	24.1		33.0						832		100			0		1.8
	V	671,000	85.1		10.6			4.2					2,000		100			0		0.5
	VI	343,000	89.2				10.7						1,920		100			0		0.7
	VII	362,000	70.0	0.4	22.7		6.9						98		100			0		0.6
	VIII	755,000	84.5	0.5	12.0		3.0						12,600		100			0		4.0

	IX	115,000	69.3		19.6		6.0			5.0		17,600		100			0		6.0
	X	755,000	89.6	0.2	2.1	5.6	0.4			2.1		596		100			0		4.2
	XI‡	(Contaminated plates)										2,980		100			0		6.8
	XII	635,000	92.3	0.7	0.3		6.7					44		100			0		3.6
	XIII	900,000	93.6		2.7		1.6			2.1		216		100			0		4.5
	XIV	181,000	78.6	0.45	1.4		14.4					1,450		100			0.22	100	4.6
	XV	206,000	91.4		4.6						4.0	285		88.9		11.1	0		4.6
	XVI	710,000	67.5	4.9	21.7		5.9					28		100			0		5.4
No. 3	I*	1,710,000	81.1	13.3	2.2		2.2					1,390		100			0		4.5
Age 35	II†	821,000	78.0	0.65	4.2	2.7	12.4	2.0				1,200		100			0		2.6
Advanced	III	260,000	76.6	3.8	10.9		8.7					1,760		100			0		4.0
pyorrhea	IV	1,870,000	88.3		5.5		5.5			0.5		1,780		100			0.18	100	0.5
	V	1,500,000	90.4				5.4			4.2		8,800		100			0		6.1
	VI	500,000	91.4			3.9	4.3			0.4		430		100			0		5.7
	VII	488,000	90.8	0.1	5.7	0.7	2.7					750		100			0		4.1
	VIII	575,000	87.4	2.1		2.8					7.7	190		100			0		5.1
	IX	778,000	81.0	1.9			16.1					943		100			0		5.6
	X	1,350,000	99.0	0.2		0.6					0.9	56		100			0		9.0
	XI‡	953,000	95.1	0.2	2.7		2.0					49		100			0		8.8
	XII	1,572,000	78.8		8.1		5.0					161		99	1.0		0		10.3
No. 8	I*	11,200	90.9				9.1					34		100			0		0
Age 24	II	24,500	86.9			13.7						20		100			0		0
Normal	III§	3,830,000	98.6	1.4								200		100			0		0.8
mouth	IV	597,000	43.5	2.8	16.9		36.5					69		100			0		0.4
	V	1,340,000	21.7		32.2		45.8					16		100			0		0
	VI	43,600	77.7		22.2							14	40	60			0		0.1
	VII	171,000	89.1	5.4		5.4						4,100		100			0		0.1
	VIII	482,000	68.5		0.56		21.1				9.6	72		100			0		0
	IX	20,200	50.0			40.0						182		100			0		2
	X	562,000	87.9	0.9	2.2		7.0		1.9			77		100			0		0.4
	XI	342,000	74.5			10.4	15.1					32		100			0		0.5
	XII	242,000	68.5	4.8	10.8	3.4	5.2				7.3	1,060		100			0		0.6
	XIII	582,000	88.7	5.0	1.1		4.1				1.1	15,200		100			0		2.1
	XIV	1,040,000	95.6		0.4						4.0	730		100			0		2.5

* Prison diet.
† Carbohydrate diet.
‡ Protein diet.
§ Balanced diet.

TABLE 3: First part. Group I

BLOOD AGAR-PLATES (AEROBIC)

Case no. and age	No. exam	Degree of detachment in mm.	pH of saliva.	Amount of debris in mgm.	Average number of organisms per mgm.	Average per cent streptococci			Average per cent plate incidence						Streptococci							
						Alpha	Beta	Gamma	Gram − diplococci	Small gram + rods	Staph.	Mic. tetrag.	Slender threads	Large gram + cocci	S. saliv. 2.1	S. saliv. 2.2	S. mitis 1.1	S. mitis 1.2	Inulin +	S. equinus	S. ignavus	Non-lactose inulin ferm.
No. 4 Age 24 Normal mouth	I*	0.54	7.4	66.8	57,500	95.2		5.8														
	II		7.4	64.7	51,000	96.9		3.1														
	III†		7.7	12.9	1,260,000	82.9				15.0							+	++		+		
	IV		7.6	14.0	1,870,000	93.2			6.8						+							
	V		7.8	67.8	951,000	50.0			11.7	39.3								+++				
	VI		7.5	54.9	1,730,000	83.5			16.4							+						
	VII		7.4	62.9	1,720,000	82.4			13.0	2.2								+++				
	VIII		7.7	43.7	947,000	77.4			2.7	19.8												
	IX		7.4	57.8	479,000	87.9			6.3	5.6												
	X		7.4	27.0	796,000	83.2			10.0	31.3				5.3	+		++		+			
	XI		7.4	45.0	1,365,000	74.9			9.0	12.2			1.8	1.8	+			++	+			
	XII‡		7.2	15.7	547,000	71.1		1.2	5.4	11.1				11.1								
	XIII		6.9	56.9	309,000	86.4			13.5						+	++						
	XIV		7.4	55.3	247,000	70.7			8.2	15.3				1.7								
	XV		6.9	37.9	535,000	83.1		5.3	1.5	10.0							+	+		++		
	XVI		7.1	34.2	453,000	78.7		2.6	1.3	8.1			0.6	7.1					+			
	XVII		7.5	59.8	571,000	74.0		16.3	5.2	3.0	1.2					++	+	+				
No. 6 Medium pyorrhea	I*	5.5	7.2	132.4	62,700	93.9																
	II		7.2	88.9	Plates broken													+				
	III†		7.5	78.0	710,000	89.7				10.2												
	IV		7.3	95.7	1,480,000	72.2			8.3	19.5							+++					
	V		7.2	97.2	1,280,000	81.4			11.8	5.1	1.4											
	VI		6.9	67.1	464,000	88.6			1.5	7.7					+							
No. 5 Medium pyorrhea	I*		7.2	91.4	277,000	90.5		2.2	1.3	5.8								+		+		
	II		7.2	40.9	85,300	71.1				20.0									++			
	III†		7.3	59.5	411,000	78.0			5.0	13.2	2.4						+				+	
	IV			51.9	Plates contaminated																	
	V		7.3	65.8	674,000	62.7			6.7	29.4			1.1									+
	VI		7.1	40.8	120,000	73.9		7.7	12.5	5.9							+++		+	+		
	VII		6.9	66.8	600,000	91.8			1.8		6.2								+			
	VIII		6.9	88.9	438,000	85.0		7.7	5.0	1.0				2.0	+		+++		+	+		
	IX‡		6.4	83.3	577,000	98.3					1.6					+		+		++		
No. 7 Advanced pyorrhea	I*		7.9	145.1	441,000	95.3																
	II		7.9	159.6	858,000	94.2				4.8	1.0					++	+	++				
	III†		7.6	123.1	311,000	68.5			29.1								+					
	IV		7.6	100.6	570,000	80.7		1.0	8.7	6.3			3.2			+		++	+		+		
	V		7.9	82.8	239,000	96.2	0.7	0.35			2.2				+			++	+			
	VI		7.6	87.9	409,000	95.4											+	+	+			
	VII		7.9	89.0	844,000	68.3	3.4		5.0	23.3								+	+			
	VIII		7.9	85.6	411,000	78.3		10.5	11.2								+	+	+			
	IX		8.3	69.5	516,000	82.5		8.4						8.4				+	+			
	X‡		8.2	90.9	489,000	88.7	0.6	1.4		3.2			0.6				++	+	+			
	XI		7.5	97.5	843,000	53.4	2.9			41.5					+						+	
	XII		7.3	82.4	859,000	65.0				35.0								+	+			

* Prison fare. † Protein diet. ‡ Carbohydrate diet.

TABLE 3: Second part. Group I, continued

Case no. and age	No. exam.	SERUM OR ASCITIC-FLUID AGAR-PLATES (ANAEROBIC)											ACID (pH 4.8–5.0) AGAR-PLATES (ANAEROBIC)					GLUCOSE AGAR-PLATES (ANAEROBIC)		
		Average number of organisms per mgm.	Average per cent plate incidence										Average number of organisms per mgm.	Average per cent plate incidence				Heated material		Stained slides; % Spirals
			Strep.	Lepto. buccalis	Mic. gasgenes	Small gram + rods	Large gram − diplococci	Staph.	Slender gram − threads	Large gram + cocci	Slender gram + threads			Strep.	Small gram + rods	Large gram fila.	Thin gram + threads	No. of organisms per mgm.	Gram + aerobic rods	
No. 4 Age 24 Normal mouth	I*	73,300	97.9					2.1					980		39.6	60.3		0		7.5
	II	185,000	83.3					16.7					187					0		11.3
	III†	1,620,000	100.0										0					0		2.8
	IV	1,920,000	32.9		5.1	62.0							54	90.5	9.4			0		1.8
	V	1,150,000	87.1		2.1	10.8							94	19.6	80.4			0		5.5
	VI	1,680,000	65.2	2.9	5.9	29.6							251		100			0		5.2
	VII	693,000	92.1	6.5			1.3						257		100			0		5.4
	VIII	855,000	72.7	6.3		0.9	4.5	15.4					6.8		100			0		3.0
	IX	482,000	74.5	4.6	12.8	8.1							74	74	25.9			0		5.5
	X	1,538,000	77.3	2.4	11.5	8.8			0.4				20	92.4	5.7	1.8		0		4.8
	XI	881,000	93.4	1.3	1.3	3.6							13	14.2	85.8			0		2.2
	XII‡	280,000	95.7			4.3							58		100			0		1.9
	XIII	239,000	92.2	1.7	0.5	1.7	3.8		2.5				5,800		100			0		2.0
	XIV	305,000	83.4	1.0	8.6	4.0			3.0				56		61.2	38.7		0		4.3
	XV	784,000	86.5	4.9	3.2	2.9							203		100			0		2.9
	XVI	288,000	89.9	1.1	6.0	1.1	1.8				3.9		160		100			0		2.2
	XVII	463,000	85.8		10.3								128		99.5	0.5		0		3.6
No. 6 Medium pyorrhea	I*	40,500	100			0.75							44.4		75	25		0		18.2
	II	595,000	99.2			1.0							51	100				0		10.1
	III†	548,000	99.0			1.8	1.1						470	1.6	98.3			0		2.5
	IV	1,780,000	96.9			2.9	20.5	5.8					1,040	58.0	41.9			0		13.0
	V	895,000	70.5				8.2						2,190		100					8.0
	VI	597,000	91.8						5.6				54,600		98.4		1.6	0.1	100	5.3
No. 5 Medium pyorrhea	I*	405,000	86.1	1.0	5.2	2.0	5.5			3.4			9,540		98.1	1.9		0		2.2
	II	640,000	61.5	2.1	5.0	22.3			5.0	1.2			2,740		100			0		0.2
	III†	332,000	87.1	2.6	1.5	2.9	1.5		3.5				1,250	5.8	94.1			0		4.9
	IV	1,795,000	94.5		0.5								840		100			0		5.1
	V	603,000	88.1	0.1	6.8	5.0	3.3			1.4			720		55.3	44.6		0		7.8
	VI	210,000	89.7	2.2	2.1	2.1	0.8				2.0		88		100					0.7
	VII	791,000	89.1	0.6	6.4	0.8					3.4		4,048		20.4	79.6				5.4
	VIII	209,000	73.5	2.1	12.4	8.6		2.8	2.8				9,095		8.5	91.5				6.7
	IX‡	246,000	77.3	6.4	8.2	2.3														5.7
No. 7 Advanced pyorrhea	I*	580,000	47.6	1.7	6.1	44.6							960		38.6	61.3		0.06	100	7.0
	II	513,000	68.7			8.4		22.9					180		90	10		0		9.1
	III†	597,000	67.3		10.2	16.7			5.8				2,020		100			0		8.1
	IV	1,490,000	66.1	1.0	10.4	16.1			6.4				11		100			0		7.7
	V		No cultures										73	14.3	14.4	71.2		0.01	100	3.8
	VI		Plates contaminated										6.8		100			0		0.3
	VII	856,000	96.7	0.2	2.1				1.0				0					0		8.7
	VIII	350,000	80.9		2.7	4.0	6.2		6.2				1.7	100				0		7.6
	IX	894,000	95.6	0.7	2.3						1.3		5.0		100			0		4.9
	X‡	740,000	88.9		7.7	3.4							21		100			0.11	100	3.7
	XI	1,450,000	95.4		2.0	3.5					1.1		3		100			0		7.0
	XII	1,572,000	96.3								1.7		3		78	22				6.1

* Prison fare. † Protein diet. ‡ Carbohydrate diet.

TABLE 4: First part. Group II

BLOOD AGAR-PLATES (AEROBIC)

Case no. and age	No. exam.	Av. degree detachment in mm.	pH of saliva	Amount of debris in mgm.	Average number of organisms per mgm.	Average per cent streptococci			Average per cent plate incidence							Streptococci						
						Alpha	Beta	Gamma	Gram − diplococci	Small gram + rods	Large gram + threads	Staph.	Mic. tetrag.	Gram − rods	Slender gram − threads	S. saliv. 2.1	S. saliv. 2.2	S. mitis 1.1	S. mitis 1.2	Inulin +	S. equinus	S. ignavus
No. 9	V*	5.3	7.2	31.1	980,500	50.4			15.6	27.2	0.35			5.2	3.6	+++			+	+		+
Age 28	VI†		7.4	61.4	1,128,000	47.2			38.1	11.1												
	VII†		6.5	92.6	1,300,000	86.1			10.6	3.3									+	+		
	VIII		6.7	109.8	704,000	54.5		0.3		38.8					1.6					+	++	
No. 10	III*	3.0	7.4	62.1	548,000	84.8			12.1	3.0							+	+++	+			
Age 31	IV		7.4	77.0	766,000	55.5		6.5	38.0							+			+	+		
	V		7.5	98.1	310,000	87.8			10.6													
	VI†		7.7	53.9	912,000	75.2			0.6	20.2					1.5	+					+	
No. 11	II	1.2	7.1	14.4	470,000	82.8						17.1										
Age 22	III		7.5	34.4	1,106,000	86.9				6.5		2.1		4.3		++	++		+			+
	IV		7.6	33.2	1,480,000	73.2			12.5	14.2										+		
No. 12	II*	18.8	6.9	74.6	655,000	88.5	0.16	3.4	0.95			0.95					+++		++	+		
Age 37	III		7.2	71.4	1,195,000	54.9			5.3	39.7									++			
	IV†		7.4	50.9	690,000	55.5		3.0	13.4	2.1				3.8			+++		++	+	+	+
No. 13	III*		7.4	41.0	949,000	58.7			6.1	35.1									+			
Age 26	IV		7.3	43.3	738,000	73.5				26.5							+		+	+		+
No. 14	I*		7.2	120.0	891,000	79.5			20.5			0.5					+					
Age 38	II		7.2	112.6	247,000	92.9		6.6	0.35							++			+			
	III		7.2	145.7	1,900,000	88.0		12.0												+		
No. 15	III†	10.1	6.9	51.2	102,400	93.7		3.1		3.1												
Age 38	IV		7.1	62.5	318,000	59.0			13.6	4.5				18.1	4.5				+			
	V		6.9	56.6	139,000	71.5	5.0			8.5		15.0					++					
	VI*		7.1	88.4	199,000	72.4			1.9	21.7		3.9				+	+		+	+	+	
No. 16	III†	2.2	7.1	38.3	535,500	44.2			9.7	46.0							+					
Age 34	IV		7.5	42.3	411,000	64.8		13.7		21.4										+		
	V*		7.7	60.2	46,500	39.3		3.5						57.2			+		+			
No. 17	III†	2.4	8.0	86.7	122,500	75.7			14.0	5.6		2.8							+			
Age 28	IV		6.9	45.1	134,000	70.5			18.9			8.3			2.7			+++				
	V*		7.9	47.7	341,000	78.6		15.4	4.8	1.1								+++		+		+
	VI		7.6	56.6	533,000	26.0		3.1	5.2	60.4												
No. 18	I†	8.5	7.3	102.3	244,000	82.6			1.2	10.7	5.0	1.5				++		+				
Age 37	II		6.9	116.7	115,000	99.0						0.9							+			
	III		7.1	90.0	427,000	65.8			29.6	4.0					0.9		+					
	IV		7.5	143.0	260,000	73.7			0.6	25.6						+		+				

* Carbohydrate diet † Protein diet.

TABLE 4: Second part. Group II, continued

Case no. and age	No. exam.	ASCITIC-FLUID AGAR PLATES (ANAEROBIC)									ACID (pH 4.8–5.0) AGAR-PLATES (ANAEROBIC)					GLUCOSE AGAR-PLATES (ANAEROBIC)		
		Average number of organisms per mgm.	Average per cent plate incidence								Average number of organisms per mgm.	Average per cent plate incidence				No. of org. per mgm.	Heated material	
			Strep.	Lepto. buccalis	Mic. gasogenes	Small gram + rods	Large gram − diplococci	Staph.	Slender gram + threads	Slender gram − threads		Strep.	Small gram + rods	Large gram + fil.	Yeast		Gram + spore rods; aerobic	Stained slides; per cent spirals
No. 9	V*	3,370,000	89.2		6.9					3.9	2,280		100					5.6
Age 28	VI	516,000	77.5	0.4	12.7					9.4	4.8		100			0.32	100	5.7
	VII†	2,070,000	96.1	2.2	1.7						1,120	5.4	94.6					5.8
	VIII	714,000	80.6		10.2	6.2				3.0	12		100					3.9
No. 10	III*	753,000	90.2		4.8	4.8					10,300	100						
Age 31	IV	Plates contaminated									0					0		2.1
	V	801,000	91.1	0.1	4.3	0.4			4 1		0					0		4.9
	VI†	494,000	83.2	0.3		16.5					1		100					2.1
No. 11	II	770,000	41.9		58.1						0							2.0
Age 22	III	488,000	75.0			25.0					9,760	100				0		3.3
	IV	1,720,000	94.2	0.8	0.2	2.0				1.7	66		100			0		0.1
No. 12	II*	1,360,000	98.3	0.39	0.68			0.59			3,800		98.3	1.7		0		4.8
Age 37	III	4,420,000	88.3	3.9	5.5	0.2			1.6		2,125		100			0		6.2
	IV†	923,000	85.4		4.0	9.5			1.1		1,310		100			0		6.0
No. 13	III*	47,300	96.0		0.6	2.2			0.6							0		9.6
Age 26	IV	914,000	89.9		5.7	4.4					2.8	50	50			0		0.2
No. 14	I*	2,000,000	79.3	6.6	7.8	6.3					3.4		100					0.7
Age 38	II	105,500	98.9		1.05						2,940	98.2	1.7			0		7.6
	III	361,000	81.2		14.6	0.5			3.6		1,980	31.5	68.9			0		6.8
No. 15	III†	73,200	84.6			15.4					3,300		100			0		8.5
Age 38	IV	88,000	72.3		9.2	9.2					4,060		100			0		
	V	634,000	82.9	1.6	7.6	1.9				9.2	3				100	0.16	100	5.5
	VI*	239,000	66.6	3.9		29.5				6.0	18		59.1		40.9	0		1.0
No. 16	III†	2,570,000	67.4	7.8		17.9			9.7		230		97.8		2.2	0		0.5
Age 34	IV	484,000	82.6	1.2	3.7	2.5				10.0	283	100				0		0
	V*	8,600	65.4			10.6	12		12.0		0							8.7
No. 17	III†	140,700	87.7		4.3		5.2				3	100						9.8
Age 28	IV	296,500	76.9	4.0		19.1					34	100				0		0.3
	V*	335,000	75.6	0.9	10.2	9.8			4.4		192	100				0		6.0
	VI	954,000	93.9	0.2	2.2					3.7	150		100			0		2.1
No. 18	I†	518,000	56.2	0.3		35.8			7.6		13		100			0		1.5
Age 37	II	119,500	90.7	0.7		28.5					19		100			0		7.0
	III	1,810,000	89.1	2.8					4.3	3.8	185	8.5	81.4	9.6		0		11.3
	IV	556,000	91.4	0.3	2.8	1.6			3.6	0.3	46	25	75			0		11.6
											690		100					9.8
																		13.3

* Carbohydrate diet. † Protein diet.

1. Amount of material and number of organisms

Tables 5, *6*, and *7* and *Charts 1a*, *1b*, *2a*, *2b*, are made from the data in the preceding tables and graphically indicate observed relationships. *Charts 1a* and *1b* give the logarithmic curves for the total number of organisms per mgm. for each type of medium from the men of both squads. Included on each chart are also the logarithmic curves for the actual amounts of material in mgm. taken from each man at each weekly examination. The reversal of diet per man is indicated on the curve by an appropriate symbol. Similar charts were not made for the men of Group II, because the number of exam-

TABLE 5

Average amounts of debris in milligrams (Group I)

DIET	CASE NO. 1	CASE NO. 2	CASE NO. 3	TOTAL AVERAGE FOR THE THREE MEN	DIET	CASE NO. 4	CASE NO. 5	CASE NO. 7	TOTAL AVERAGE FOR THE THREE MEN	TOTAL AVERAGE FOR ALL MEN ON THE TWO DIETS	
Initial diet carbohydrate	22.6	61.7	109.3	64.5	Initial diet protein	42.6	62.2	91.2	65.3	Carbohydrate	64.9
Changed to protein	21.3	51.0	84.2	52.1	Changed to carbohydrate	43.3	53.3	90.2	62.2	Protein	58.7

inations per man was insufficient. *Charts 2a* (Group I) and *2b* (Group II) show the average amount of material in mgm. from each man (left) arranged according to the degree of mouth involvement, and also the total average for each group (right). *Table 5* gives the average number of organisms per mgm. in the three different media for the men of Group I on the three different diets. *Table 6* shows the average number of organisms per mgm. found on the acid agar-plates. This table compares the amounts obtained from the men having similar mouth conditions. The average amounts of debris in mgm. from the six men in Group I are given in *Table 7*, to indicate whether the amounts varied appreciably with the diets regardless of the degree of detachment.

In examining these different charts and tables (*Charts 1a, 1b, 2a, 2b, Tables 2–7*) several points of interest are manifested. It may be noticed that there is great uniformity in the amounts of material derived from each individual, and from the groups, representing each type of mouth condition. This is indicated in the logarithmic curves in *Charts 1a, 1b*, and particularly *Charts 2a* and *2b*, which show that the men with normal mouths and those with medium or with advanced pyorrhea may be roughly divided into these corresponding groups according to the amounts of debris obtained. This is especially evident when the averages are taken for the different men (right side, *Charts 2a, 2b*). The more advanced the case the greater the amount of debris around the teeth, as one would naturally expect where there are many deep pockets.

A change of diet from carbohydrate to protein or vice versa does not seem to alter appreciably the average amounts for the individual or for the group, regardless of the mouth condition, as shown in *Table 5*. While the amounts may be slightly greater on the carbohydrate fare, especially when it is the initial diet, yet there are no very striking differences. The averages for the men (Group I) with an initial carbohydrate diet were 64.5 mgm. on the carbohydrate; 52.1 mgm. on the protein. For the men with the initial protein diet, they were 65.3 mgm. on the carbohydrate; and 62.2 mgm. on the protein. The total average of the amounts for all these men when on the carbohydrate diet was 64.9 mgm.; when on the protein, 58.7 mgm.

The same general type of curve for the weekly amounts of debris, with one exception, seems to hold for all the men (Group I, *Charts 1a, 1b*), but the more advanced the pyorrhetic condition the larger the amounts of material. In the case of No. 1, however, the curve runs up and down most regularly, high and low amounts alternating within a range of 1 to 1.5. There is no apparent explanation for this irregular behavior.

The curves for the number of organisms per mgm. (Group I, *Charts 1a, 1b*) for all the men of both diet squads seem to follow fairly comparable lines for both the aerobic blood agar-plates and the anaerobic ascitic-fluid agar-plates, probably because of the large percentage of streptococci under each condition. No striking differences are noticed when the diets are reversed. There is, however, a tendency for an

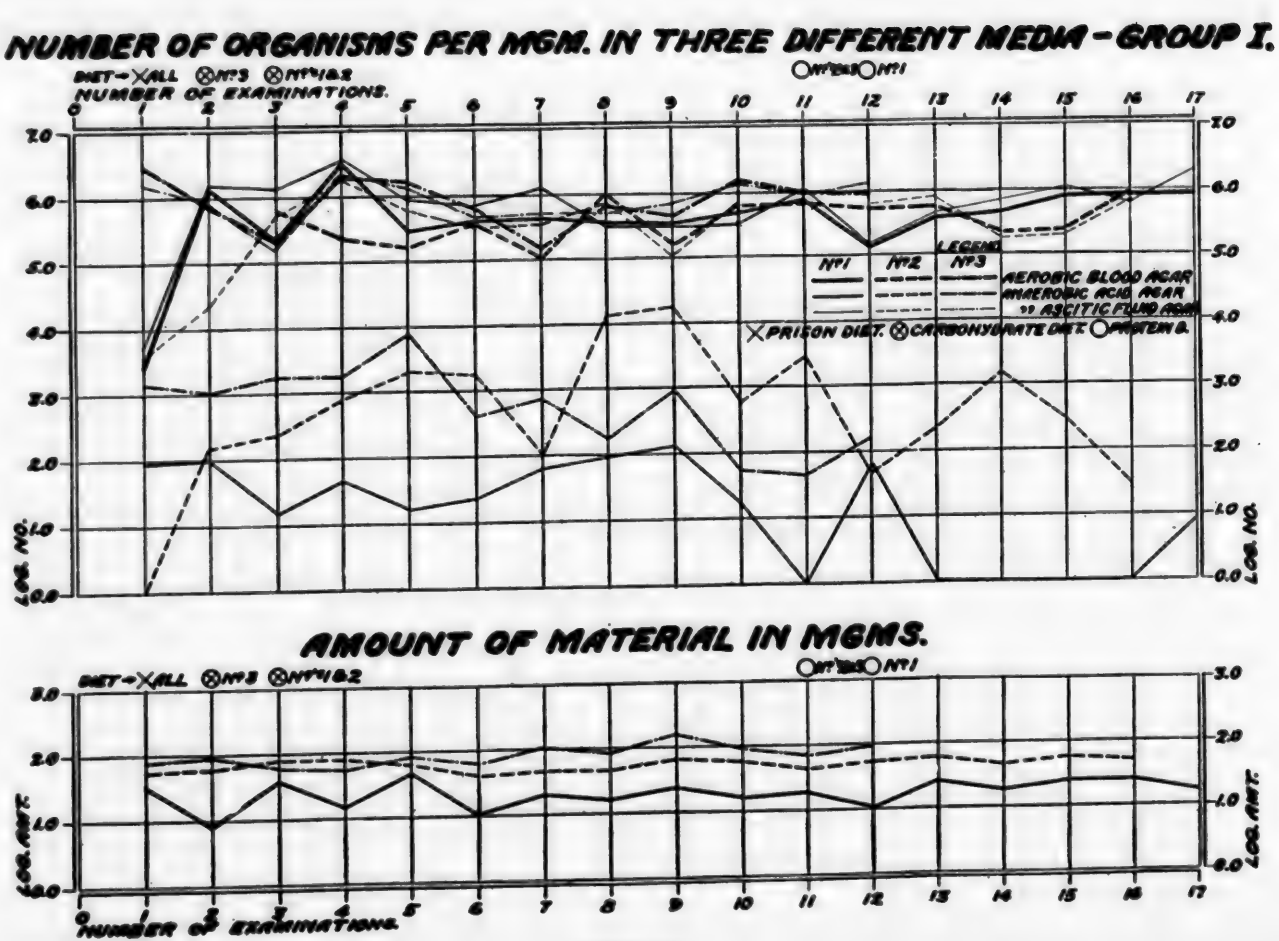

NUMBER OF ORGANISMS PER MGM. IN THREE DIFFERENT MEDIA - GROUP I.
DIET → ×ALL ⊗N°3 ⊗N°1&2
○N°1&2 ○N°1
NUMBER OF EXAMINATIONS.
LOG. NO.
LEGEND
N°1 N°2 N°3
AEROBIC BLOOD AGAR
ANAEROBIC ACID AGAR
" ASCITIC FLUID AGAR
×PRISON DIET. ⊗CARBOHYDRATE DIET. ○PROTEIN D.
AMOUNT OF MATERIAL IN MGMS.
LOG. AMT.
NUMBER OF EXAMINATIONS.

CHART 1a

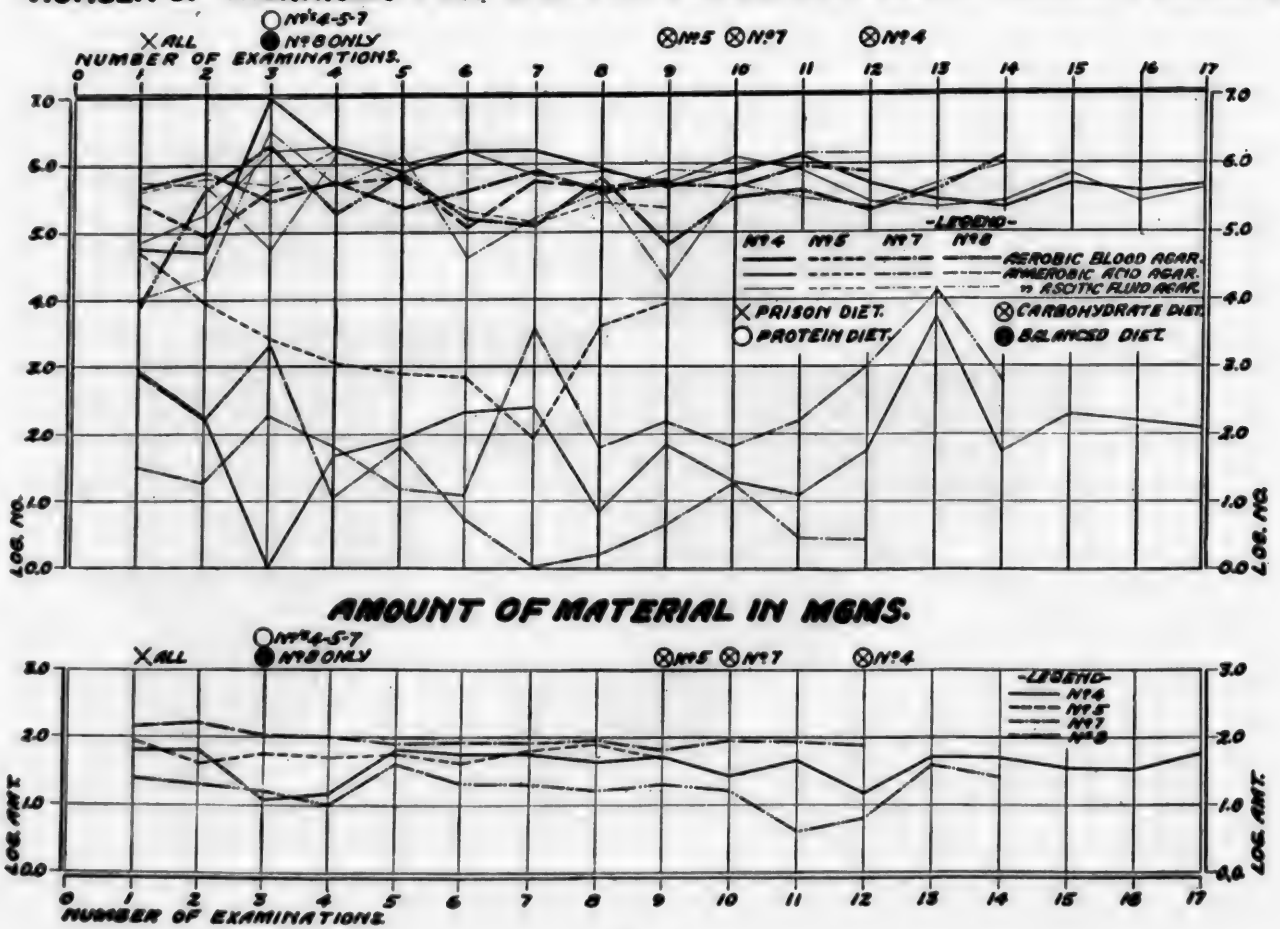
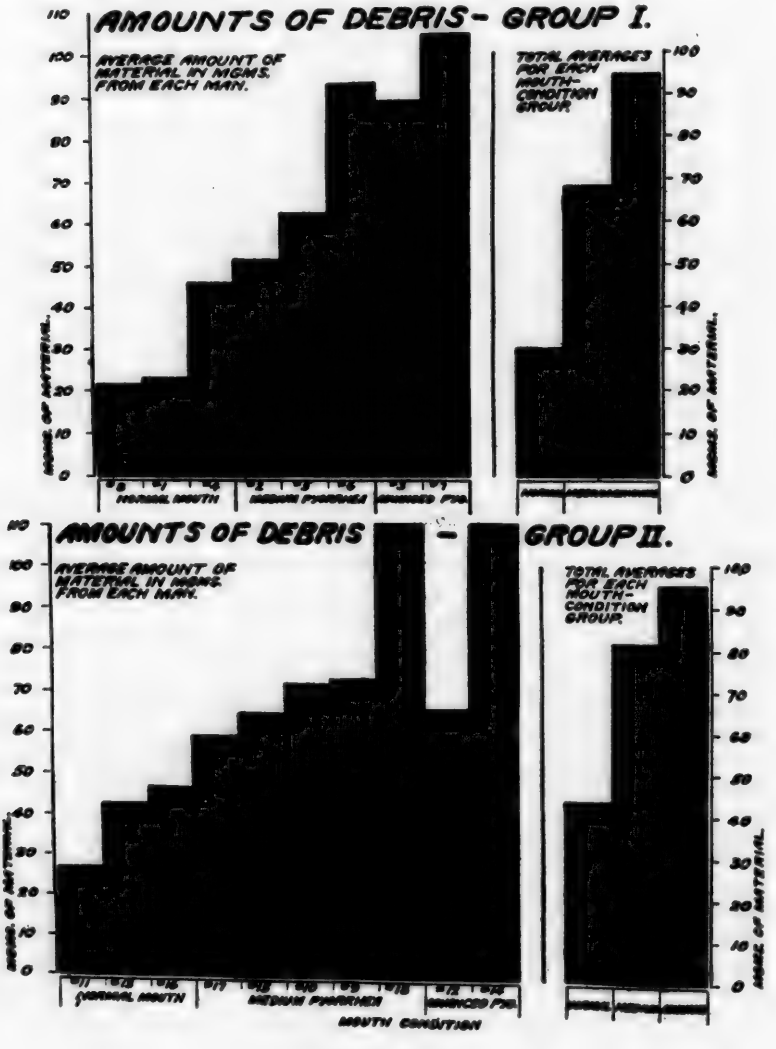

NUMBER OF ORGANISMS PER MGM. IN THREE DIFFERENT MEDIA - GROUP I.
ALL
Nº 4-5-7
Nº 8 ONLY
Nº 5
Nº 7
Nº 4
NUMBER OF EXAMINATIONS.
LOG. NO.
-LEGEND-
Nº 4
Nº 5
Nº 7
Nº 8
AEROBIC BLOOD AGAR.
ANAEROBIC ACID AGAR.
vs ASCITIC FLUID AGAR.
PRISON DIET.
PROTEIN DIET.
CARBOHYDRATE DIET.
BALANCED DIET.
AMOUNT OF MATERIAL IN MGMS.
LOG. AMT.
NUMBER OF EXAMINATIONS.

Chart 1b

increase in the number of organisms per mgm. from the men having the greatest degree of gingival detachment.


AMOUNTS OF DEBRIS - GROUP I.
AVERAGE AMOUNT OF MATERIAL IN MGMS. FROM EACH MAN.
TOTAL AVERAGES FOR EACH MOUTH-CONDITION GROUP.
NORMAL MOUTH
MEDIUM PYORRHEA
ADVANCED PYR.
AMOUNTS OF DEBRIS - GROUP II.
AVERAGE AMOUNT OF MATERIAL IN MGMS. FROM EACH MAN.
TOTAL AVERAGES FOR EACH MOUTH-CONDITION GROUP.
NORMAL MOUTH
MEDIUM PYORRHEA
ADVANCED PYR.
MOUTH CONDITION

CHARTS 2a, 2b

The curves for the organisms per mgm. from the anaerobic acid (pH 4.8–5.0) agar-plates show rather interesting variations. As one might expect, the average number is much lower in these plates than in the other media, because only the strongly aciduric bacteria could withstand the high acidity. In comparing the acid agar-graphs from the men belonging to opposite diet squads (Group I, *Charts 1a, 1b*), one notes a distinct tendency for the curves to be higher on a carbohydrate than on a protein diet, and to change when the diet is reversed. Each case did not run absolutely true in all instances, because on some days there were distinct drops in the amounts, but there seems to be some relationship between the number of aciduric organisms and the type of diet. This relationship is obvious in *Table 6*, which gives the average number of organisms per mgm. for Group I in the three media.

TABLE 6

Average number of organisms per milligram (Group I)

MEDIUM	INITIAL CARBOHYDRATE DIET	PROTEIN DIET, AFTER CHANGING FROM CARBOHYDRATE	INITIAL PROTEIN DIET	CARBOHYDRATE DIET AFTER CHANGING FROM PROTEIN	BALANCED DIET
Aerobic blood agar	711300	701300	739600	587300	615300
Anaerobic ascitic fluid agar	915100	923300	863000	631000	754200
Anaerobic acid agar (pH 4.8–5.0)	2090	310	822	3390	1816

Here it is seen that the average number of aciduric organisms from the seven men, after the carbohydrate diet, is from four to six times as great as the number of organisms present after the protein diet. The man on the balanced meals had a greater preference for carbohydrate food than for protein. While he had both to choose from, he evidently favored more carbohydrate than protein, as evidenced by the large average number of aciduric organisms.

The same relationship expressed somewhat differently is shown for Group I in *Table 7*, which compares the average number of organisms per mgm. from the acid agar-plates for the men classed according to mouth conditions. These men had alternately been given the two types of diets. The figures for No. 8 on a balanced diet are also shown. In this table one can compare the results from the men having similar mouth conditions—No. 2 with No. 5 for instance, both having medium

pyorrhea and therefore about the same amount of debris. They both show an increase of five times as many aciduric organisms on the carbohydrate diet as on the protein, although the total average number per mgm. for both is high as compared with the men with normal mouths. The latter (Nos. 1 and 4) show an increase on the carbohydrate diet of from eleven to fourteen times the number of bacteria. This increase also holds true for No. 3 (advanced pyorrhetic), but the figures are reversed with No. 7. However, this high average for No. 7 on the protein diet is mainly due to the results of one examination—the first after going on this diet. Previously, on the prison fare the amounts were also high, so that these figures for the third week may

TABLE 7

Anaerobic acid agar (pH 4.8–5.0). Average number of organisms per mgm. per man (Group I)

MOUTH CONDITION	NORMAL			MEDIUM PYORRHEA		ADVANCED PYORRHEA	
Case number	No. 8	No. 1	No. 4	No. 2	No. 5	No. 3	No. 7
Carbohydrate diet		44	1067.5	5039.5	9095	1767.6	9
Protein diet		3	96.2	933.1	1614	70	302.5
Amount increase in organisms on carbohydrate diet		×14	×11	×5.4	×5.6	×25.2	Decrease ×33.5
Balanced diet	1816.8						

show the influence of the former diet, especially since the aciduric organisms dropped off later to very small amounts per mgm. (*Table 1b*).

In comparing the graph for No. 7 with that for No. 3, the other advanced pyorrhetic case (*Charts 1a, 1b*), it is seen that the curves tend to run in opposite directions when the men are on opposite diets, even though No. 7 may show only a small number of organisms when on the carbohydrate food. The graphs for the normal control on a balanced diet (No. 8) are shown in *Chart 1b*. The curves representing the number of organisms per mgm. follow an up-and-down course for the three different media, while that for the amount of debris is about the same as for the other men in Group I. The sharp peaks for No. 8 are probably induced by the fact that he alternated his meals between the two diet squads, having one with the protein men and the next with the carbohydrate. In this way he obtained two of the same meal types

every day, although a different two on alternate days. He was also free to choose what he preferred from the two types of fare, and consumed whatever candy he could obtain between meals.

The number of examinations for the men of Group II was insufficient to warrant chartings similar to those for Group I, but from the information given in *Table 4* it seems that comparable results were obtained in respect to the amounts of debris, these amounts having also been fairly constant for the individual and for the group in each type of mouth condition (*Chart 2b*). Concerning the aciduric organisms, the insufficient number of examinations precludes deductions from the figures for Group II, although in most cases the number was increased on the carbohydrate diet.

2. Relative proportions of the bacterial species found in the different media

(*a*) *Aerobic blood agar-plates.* The aerobic blood agar-plates did not show a very great variety of bacterial species, the same few types appearing quite regularly in varying amounts at each examination. The high dilutions of the stock suspension probably helped to eliminate the slower growing forms and those not so numerous, retaining both the most viable and those in largest numbers among the aerobes that were around the selected eight teeth of each man. Streptococci, gram-negative diplococci of the Flavus group, gram-positive diphtheroid rods, staphylococci, and large round gram-positive cocci in groups were among those most frequently encountered. No attempt was made to identify all of the latter, but they were usually grouped under the heading of large gram-positive cocci and for the most part were related to *Diplococcus crassus*. Occasional colonies of *Micrococcus pharyngicus siccus*, *Micro. catarrhalis*, and *Micro. tetragenous* were also present in small percentages.

With a few exceptions the streptococci far outnumbered all the other forms, both relatively and absolutely at every examination, for both Groups I and II. Case No. 1 on the third week and No. 3 on the second both showed larger numbers of gram-negative diplococci, while No. 8 on the fifth week, No. 16 on the first, and No. 17 on the fourth, respectively, had a predominance of diphtheroid rods (*Charts 3a*, *3b* and *6*). However, there was no particular uniformity in respect

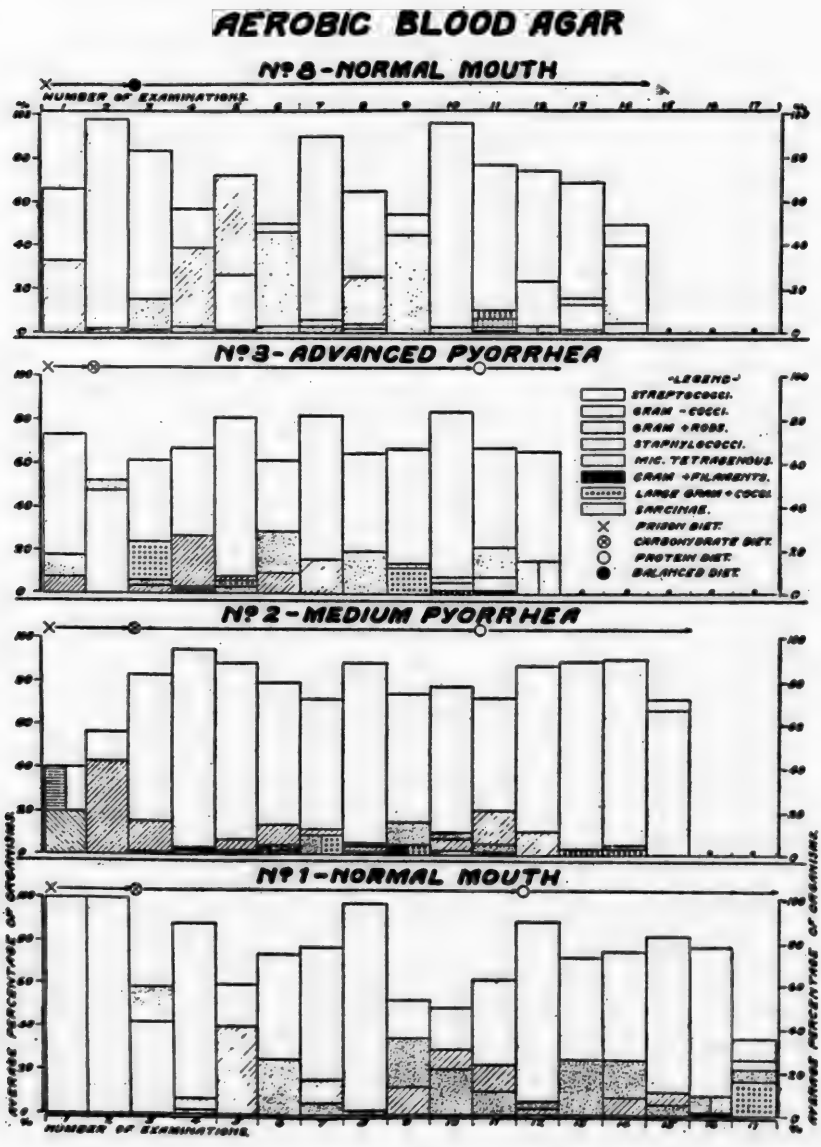

AEROBIC BLOOD AGAR
No 8 - NORMAL MOUTH
NUMBER OF EXAMINATIONS.
No 3 - ADVANCED PYORRHEA
LEGEND
STREPTOCOCCI.
GRAM - COCCI.
GRAM + RODS.
STAPHYLOCOCCI.
MIC. TETRAGENUS.
GRAM + FILAMENTS.
LARGE GRAM + COCCI.
SARCINAE.
PRISON DIET.
CARBOHYDRATE DIET.
PROTEIN DIET.
BALANCED DIET.
No 2 - MEDIUM PYORRHEA
No 1 - NORMAL MOUTH
AVERAGE PERCENTAGE OF ORGANISMS.
NUMBER OF EXAMINATIONS.

CHART 3a

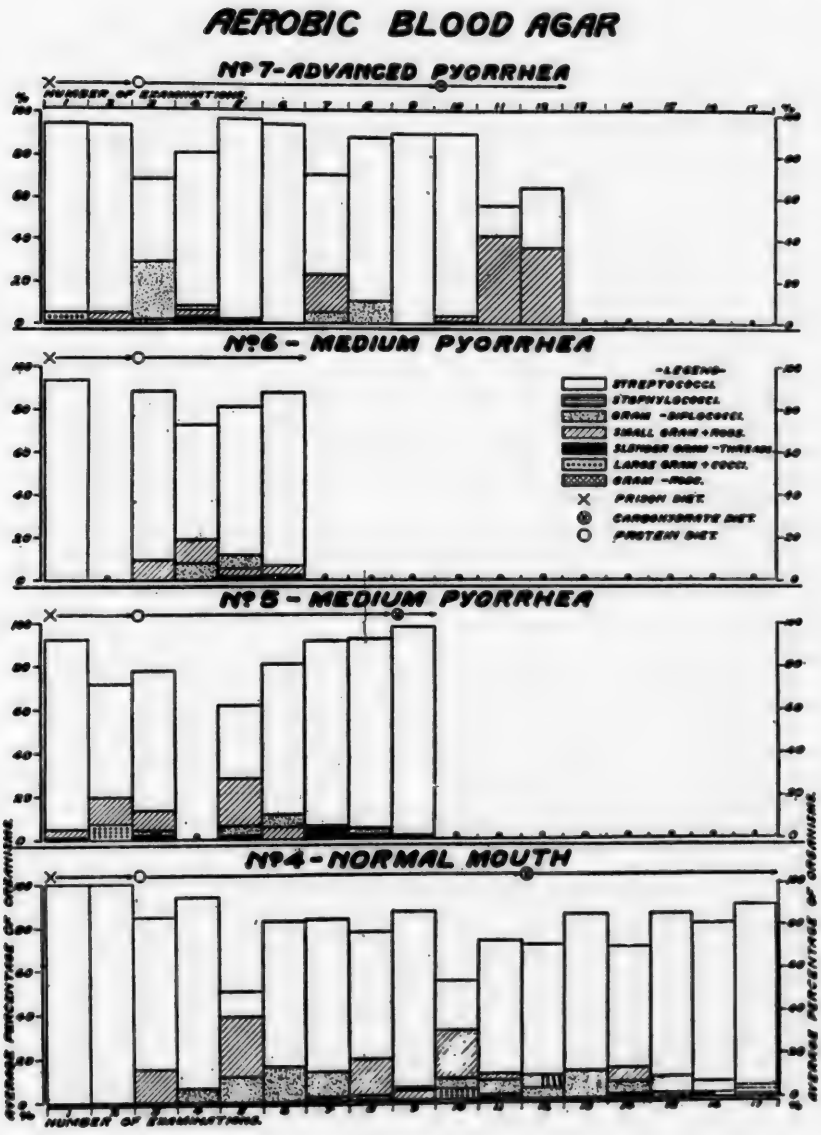

AEROBIC BLOOD AGAR
Nº 7-ADVANCED PYORRHEA
NUMBER OF EXAMINATIONS.
Nº 6 - MEDIUM PYORRHEA
-LEGEND-
STREPTOCOCCI
STAPHYLOCOCCI
GRAM -DIPLOCOCCI
SMALL GRAM + RODS.
SLENDER GRAM -THREADS
LARGE GRAM + COCCI.
GRAM -RODS.
PRISON DIET.
CARBOHYDRATE DIET.
PROTEIN DIET.
Nº 5 - MEDIUM PYORRHEA
Nº 4 - NORMAL MOUTH
AVERAGE PERCENTAGE OF ORGANISMS.
NUMBER OF EXAMINATIONS.

CHART 3b

TABLE 8

Average percentage incidence of organisms on the aerobic blood agar-plates

GROUP	CASE NO.	PRISON DIET							CARBOHYDRATE DIET							PROTEIN DIET							BALANCED DIET						
			Streptococci							Streptococci								Streptococci								Streptococci				
		No. of exam.	Av. % alpha	Av. % beta	Av. % gamma	Av. % gram − diplococci	Av. % gram + rods	Av. % staph.	No. of exam.	Av. % alpha	Av. % beta	Av. % gamma	Av. % gram − diplococci	Av. % gram + rods	Av. % staph.	No. of exam.	Av. % alpha	Av. % beta	Av. % gamma	Av. % gram − diplococci	Av. % gram + rods	Av. % staph.	No. of exam.	Av. % alpha	Av. % beta	Av. % gamma	Av. % gram − diplococci	Av. % gram + rods	Av. % staph.
I	1	2	100	0	0	0	0	0	9	64.6	0.1	2.1	18.1	14.5	0	6	63.8	0.2	5.6	15.3	11.1	0.05							
	2	1	40	0	0	0	0	20.0	8	75.1	0.4	2.8	5.9	11.9	1.0	6	75.9	0.5	2.3	3.4	14.7	1.0							
	3	1	73.5	0	0	18.6	7.9	0	9	66.2	0.08	2.5	15.1	7.9	0	2	62.4	0	0	18.6	11.7	0							
	4	2	91.3	0	4.2	0	0	0	9	76.1	0	4.2	8.4	13.9	0	6	77.2	0	0	5.8	6.2	0.6							
	5	2	80.8	0	1.1	0.6	12.9	0	6	78.2	0	3.0	6.2	9.9	1.7	1	98.3	0	0	0	0	0.8							
	6	1	93.9	0	0	0	0	0								4	82.9	0	0	5.4	10.6	0.3							
	7	2	94.7	0	0	0	2.4	0.5	7	81.4	0.5	2.8	10.5	4.2	0.3	3	69.0	1.1	0.4	0	26.5	0							
	8	2	64.4	0	1.3	0	33.3	0.8															12	62.1	0	2.0	15.3	15.8	0
Total		13	80.3	0	0.8	2.4	7.0	2.6	48	73.6	0.18	2.9	10.7	10.3	0.5	24	75.6	0.4	1.1	6.9	11.5	0.39	12	62.1	0	2.0	15.3	15.8	0
II	9								2	48.8	0	0	26.8	19.1	0	2	70.3	0	0.1	5.2	21.0	0							
	10								3	76.0	0	2.1	20.2	1.0	0	1	75.2	0	0	0.6	20.2	0							
	11								2	84.8	0	0	0	3.2	9.6	1	73.2	0	0	12.5	14.2	0							
	12								2	71.7	0.08	1.7	3.1	19.8	0.4	1	55.5	0	3.0	13.4	2.1	0							
	13								2	66.1	0	0	3.0	30.3	0														
	14								3	86.8	0	6.2	6.9	0	0.1														
	15								1	72.4	0	0	1.9	21.7	3.9	3	74.7	1.6	1.0	4.5	5.3	5.0							
	16								1	39.3	0	1.7	0	0	0	2	54.5	0	6.8	4.8	33.7	0							
	17								2	52.3	0	9.2	5.0	30.7	0	2	73.1	0	0	16.4	2.8	5.5							
	18															4	80.2	0	0	7.8	10.0	0.6							
Total									18	66.4	0.008	2.3	7.4	13.9	1.4	16	69.5	0.2	1.3	8.1	13.6	1.3							

to organism, diet, or mouth condition, so that one should not ascribe too much importance to these irregularities. Of the streptococci, the alpha or green-producing type (Brown, 1919) was always the predominant form, interspersed occasionally with a small percentage of gamma (anhemolytic) and a still smaller number of beta (hemolytic) streptococci. Out of ninety-one separate examinations, 71.2 percent of the organisms on the blood agar-plates were alpha streptococci, 1.4 percent gamma, and 0.13 percent beta. These averages were obtained from the figures in *Table 8*, which gives the average percentage incidence of the organisms most frequently found on the aerobic plates, grouped according to the number of examinations for the men on each kind of diet. There seems to be no predominance of any one organ-

TABLE 9

Incidence of beta streptococci

CASE NO.	NO. OF EXAMINATIONS	BETA STREP. NO. OF ISOLATIONS	TYPES OF BETA STREPTOCOCCI			
			S. equi		S. pyogenes	
			No. of isolations	Per cent	No. of isolations	Per cent
1	17	1	1	100	0	0
2	16	6	0	0	6	100
3	12	1	1	100	0	0
7	12	2	0	0	2	100
19	16	7	3	42.8	4	57.2
Total.....	73	18	5	48.5	12	51.2

ism on any one diet for either Group I or II. In fact the total averages show a very close relationship in percentage for each species on each diet. In Group I alpha streptococci averaged 73.6 percent on the carbohydrate diet and 75.6 percent on the protein; in Group II, 66.4 percent on the carbohydrate and 69.5 percent on the protein. In the same manner within a certain range, corresponding figures for all the organisms are given for the men of each diet squad in each group. No. 8 on the balanced diet gave the smallest percentage of alpha streptococci (62.1), while the men in Group I on the prison fare gave the highest (80.3). The diet apparently has very little effect upon the frequency of the different aerobic forms growing in this medium.

The very small average percentage of the hemolytic or beta strepto-

cocci from any of the men seems to indicate a minor rôle for these forms in pyorrhea, while the high average of the alpha type may be significant. The hemolytic type was isolated consecutively from only two men (Nos. 2 and 19), and was occasionally found from only three others (Nos. 1, 3 and 7). It was isolated eighteen times out of 73 different examinations of these five men, two of whom (Nos. 1 and 19) had normal mouths and the other three pyorrhea (Nos. 2, 3 and 12). Only two forms of hemolytic streptococci were encountered, *S. equi* and *S. pyogenes*, of which the former averaged 48.5 percent and the latter 51.2 percent, respectively, of the total number of isolations (*Table 9*).

Alpha streptococci were present in varying percentages at every examination, and were grouped by their carbohydrate reactions into various sub-types according to the Brown-Holman classification. The incidence of the different identified types is given in *Table 10* for Groups I and II, showing the number of examinations for each man, together with the number of isolations and the percentage of alpha streptococci for each sub-type. The alpha forms fell into the general groups *S. salivarius*, *S. mitis*, and *S. equinus* or non-lactose fermenters. The first two forms are further subdivided according to their behavior toward raffinose; *S. salivarius* 2.2 and *S. mitis* 1.2 fermenting raffinose, and *S. salivarius* 2.1 and *S. mitis* 1.1 forming no acid from this sugar. Under the heading of inulin fermenters in the table are included the strains that correspond to *S. mitis* 1.1 in all carbohydrate reactions, but also ferment inulin. They all show chain formation and are insoluble in bile. *S. equinus* ferments only sucrose and salicin, not lactose, mannite, or inulin. *S. ignavus* ferments sucrose, but not the other sugars. All the types that do not ferment lactose are grouped in *Table 10* under the heading "non-lactose fermenters." In 103 different examinations and 197 isolated alpha streptococcus colonies from Group I, there is an average incidence of 56 or 25.7 percent for *S. salivarius* (2.2 and 2.1), 90 or 46.9 per cent for *S. mitis* (1.2 and 1.1), 28 or 14 percent for the inulin fermenters, and 23 or 11 percent for the non-lactose fermenters. For Group II, in 34 different examinations and 78 isolations of alpha streptococci, the average incidence is 27 or 35.5 percent for *S. salivarius*, 25 or 32.9 percent for *S. mitis*, 11 or 14.1 percent for the inulin fermenters and 15 or 17.8 percent for

the non-lactose fermenters. *S. mitis* predominated in Group I, while *S. salivarius* are slightly more prevalent in Group II, provided the inulin fermenters are not included with the mitis organisms. The non-lactose fermenters are less prevalent and run in about the same pro-

TABLE 10

Incidence of the different alpha streptococci in Groups I and II

GROUP	CASE NO.	NO. OF EXAM.	S. SALIVARIUS 2.1. NO. OF INOC.	PER CENT OF ALPHA STREP.	S. SALIVARIUS 2.2. NO. OF INOC.	PER CENT OF ALPHA STREP.	S. MITIS 1.1. NO. OF INOC.	PER CENT OF ALPHA STREP.	S. MITIS 1.2. NO. OF INOC.	PER CENT OF ALPHA STREP.	INULIN FERMENTERS. NO. OF INOC.	PER CENT OF ALPHA STREP.	NON-LACTOSE FERMENTERS. NO. OF INOC.	PER CENT OF ALPHA STREP.	TOTAL NO. ALPHA STREP. ISOLATED
I	1	17	8	25.8	4	12.8	8	25.8	8	25.8	1	3.2	2	6.3	31
	2	16	7	21.2	6	18.1	9	27.2	7	21.2	2	6.0	2	6.0	33
	3	12	6	26.0	2	8.5	4	17.3	5	21.3	4	17.3	2	8.5	23
	4	17	4	11.7	8	23.5	5	14.7	10	14.7	3	8.7	4	11.7	34
	5	9	1	5.2	1	5.2	4	21.0	2	10.5	6	31.5	5	26.3	19
	6	6	1	16.6			4	66.6	1	16.6					6
	7	12	2	6.2	3	9.3	6	18.7	7	21.8	8	25.0	6	18.7	32
	8	14	2	10.5	1	5.2	2	10.5	8	42.1	4	21.0	2	10.5	19
Total		103	31	15.4	25	10.3	42	25.2	48	21.7	28	14.0	23	11.0	197
II	9	4	4	36.3					2	18.1	2	18.1	3	27.2	11
	10	4	2	20.0	1	10.0	3	30.0	2	20.0	1	10.0	1	10.0	10
	11	3	2	28.5	2	28.5			1	14.2	1	14.2	1	14.2	7
	12	3			3	30.0			3	30.0	2	20.0	2	20.0	10
	13	2			1	20.0			2	40.0	1	20.0	1	20.0	5
	14	3	2	33.3	1	16.6			2	33.3			1	16.6	6
	15	4	1	11.1	2	22.2			1	11.1	2	22.2	3	33.3	9
	16	3			2	40.0			2	40.0	1	20.0			5
	17	4					3	37.5	1	12.5	1	12.5	3	37.5	8
	18	4	3	42.8	1	14.2	2	28.5	1	14.2					7
Total		34	14	17.4	13	18.1	8	9.6	17	23.3	11	14.1	15	17.8	78

portions in both groups of men, while the inulin fermenters average the same (14 percent) for both Groups I and II. Glynn and Digby (1923) also found about the same average (12.3 percent) of inulin fermenting alpha streptococci.

The green-producing streptococci could be divided into groups according to their behavior in carbohydrate media and their colony appearance in the poured blood agar-plates. Several definite shapes of typical alpha streptococci were always present in the material from every mouth. These were designated as "rosette" (having many small projections), "mitral" or "wedge," and "smooth" or "lenticular." A fourth designation, "winged" (with quite extended projections from different sides), was used at first, but as it was difficult to differentiate

TABLE 11

Average percentage of alpha streptococci classed by colony appearance

CASE NO.	NO. OF EXAM.	AVERAGE PER CENT "ROSETTE" COLONIES	AVERAGE PER CENT "WEDGE" COLONIES	AVERAGE PER CENT "SMOOTH" COLONIES	NO. OF EXAM.	AVERAGE PER CENT "WINGED" COLONIES
1	10	44.7	22.6	31.4	1	7.3
2	10	53.0	19.1	27.1	3	2.1
3	11	46.5	11.2	38.0	5	9.1
4	9	51.2	12.3	32.3	3	8.6
5	7	57.5	14.5	25.2	1	16.6
7	10	53.0	14.9	25.8	5	12.5
8	6	48.4	6.2	44.3	1	4.0
9	4	35.8	35.3	26.6	1	8.3
10	3	56.5	16.6	26.6		
11	2	64.6	13.9	21.4		
12	2	41.8	7.8	50.4		
13	2	69.5	4.0	26.5		
14	2	59.2	2.6	38.1		
15	3	32.6	8.2	59.1		
16	2	75.1	10.8	14.0		
17	2	35.6	25.0	39.3		
18	2	53.8		46.1		
Total.....	87	51.6	13.2	33.6	20	8.5

these forms from the "rosettes," both types were later classed together (*Plate 1, figs. 7–9*). The "rosette" and the "wedge" shaped colonies would probably be classed as rough, R, and the lenticular as smooth, S (Cowan, 1924). The average percentage of the different colony formations among the alpha streptococci is given in *Table 11*. In a total of 107 different examinations, 51.6 percent of the alpha colonies were "rosette," 8.5 percent were "winged," 13.2 percent were "wedge" shape, and 33.6 percent were "smooth," the largest percentage falling on the side of the so-called "rough" colonies.

An attempt was made to determine if there was any definite relationship between colony formation and the carbohydrate reactions, but apparently there is no correlation, except that the *S. equinus* types were isolated only from smooth colonies. However, not all smooth colonies were *S. equinus*. Other forms such as *S. mitis* and *S. salivarius* also came from smooth colonies.

From the work of Cowan (1924) on beta (hemolytic) streptococci, it has been shown that smooth (S) colonies are highly virulent for laboratory animals while rough (R) are avirulent. With this idea in mind, many different colonies of alpha streptococci were isolated in this work and 18-hour glucose broth-cultures were injected intraperitoneally into mice in 1 cc. amounts. The dose was made large to eliminate non-virulent strains. Of 70 mice inoculated, 57 or 81.4 percent died within three days—two-thirds of them in less than eighteen hours. The mortality was about equally divided for the strains isolated from the three different colony types. However, there seems to be later evidence that strains, regardless of the original type, dissociate after mouse passage into a large predominance of the smooth forms. The biochemical reactions usually remained the same before and after mouse passage.

There seemed to be no uniformity in the fluctuations of the other types of organisms. The greatest variations were with the gram-negative diplococci and the gram-positive diphtheroid rods. Their relative amounts varied for all the men, regardless of mouth condition or diet, the greatest variability, however, being in the material from the men with normal mouths (*Charts 3a, 3b,* and *6*). The percentages of the different organisms from No. 8, the control on the balanced diet, showed the greatest fluctuation. At one time one form predominated and the next time the other. Kligler and Gies (1915) also found more variations in the material from normal men. *Micrococcus flavus*, Group I, fermenting glucose, levulose, maltose, and sucrose, was the form of gram-negative diplococcus most frequently encountered. It was isolated quite often from every mouth examined. The asporogenic, small, gram-positive, diphtheroid rods that were isolated usually belonged to the most highly fermentive strains of the heterogeneous group, Diphtheroids VI and IX. These organisms fermented about the same group of carbohydrates as did the Lactobacillus rods found

on the acid agar-plates. Brom-cresol-purple milk, however, was never clotted and seldom strongly decolorized, as with the latter group. *Table 12* gives the percentage of the different types among the isolated strains. In a total of 33 diphtheroid strains isolated from fifteen different individuals, 27 or 81.8 percent were Type IX, 3 or 9.0 percent Type VI, 2 or 6.0 percent Type VIII, and 1 or 3.0 percent Type I. Even though they were not always isolated, diphtheroid organisms were found at one time or another from all the men examined irrespective of diet or mouth condition (*Charts 3a*, *3b*, and *6*).

(*b*). *Anaerobic ascitic-fluid agar-plates.* In the anaerobic ascitic-fluid agar-plates, the streptococcus is again the predominating organism. Both the absolute and the relative amounts (*Tables 2*, *3*, and *4*) were always the highest for all diets and for all mouth conditions with

TABLE 12

Percentage of diphtheroid types isolated

NO. OF CASES	TOTAL NO. OF ORGANISMS ISOLATED	DIPHTHEROIDS				
15	33	Type	II	VI	VIII	IX
		Number isolated	1	3	2	27
		Percent	3.0	9.0	6.0	81.8

only a few exceptions—No. 8 on the balanced diet, No. 2 on the carbohydrate, and No. 4 on the protein diets, respectively (*Charts 4a*, *4b*). On these occasions (No. 2 in the fourth week, No. 8 in the fourth and fifth, No. 4 in the fourth) the small gram-positive rods ran parallel to the streptococci or were in greater amounts. As a rule the streptococci were not isolated from these plates after the first four or five examinations, because no strictly anaerobic forms were ever found, and the colonies were all morphologically and culturally similar to those obtained under aerobic conditions, mostly of the alpha type.

The small, pleomorphic, gram-positive rods are all classed together, and recorded in the charts and tables under that designation, although in reality several forms were often present—both diphtheroids found on aerobic plates and aciduric rods isolated from acid medium. While these two types differ in aerobic surface colony-appearance, in ability

to grow in an acid medium, and in fermentation of milk, yet since their carbohydrate reactions usually ran parallel and were strongly acid, the organisms probably had the identical effects on the mouth pabulum. *Plate 1* gives microphotographs of gram-positive rods isolated from three types of media, showing the morphological resemblances. The very small gram-negative anaerobic gas-forming organism, *Micrococcus gasogenes* (Hall and Howitt, 1925) was also found on these plates, although nearly always in symbiotic relationship with gram-positive rods, slender threads, or a gram-positive coccal form. For this reason it was very troublesome to isolate in pure culture. When purified, however, there were usually present two types of colonies—smooth or disk-shaped and three-cornered or mitral. More work in respect to the characteristics of each of these types is contemplated. The organism is one of the few we encountered that is anaerobic and does not ferment any of the usual carbohydrate media. It is always present in the mouth, and can always be found by direct inoculation of saliva or of gingival debris into tubes of deep brain medium, gas production being an indication of its presence. It was not always present on the anaerobic plates in the dilutions used, although it might be found in the brain medium inoculated with more concentrated material.

Fluffy colonies with threadlike, tangled outgrowths radiating from a slightly denser center (*Plate 1*) were often encountered on these plates. They belonged to the large, thick, gram-positive, filamentous organism, *Leptotrichia buccalis* (*Plate 1, fig. 12*). It is very slow in growing, the colonies taking about four or five days to develop, and is most difficult to subculture. Other threadlike forms were also found but they were usually gram-negative or weakly positive. They were mostly thin, slender forms, often with pointed ends, and occasionally intermixed with corkscrew-like or more open spirals. They were always in symbiotic relationship with other bacteria. According to the work of Varney (1927) these types are grouped with the fusiform bacilli. *Fig. 11* on *Plate 1* shows a microphotograph of some of these forms. *Fig. 10* represents gram-negative fusiform rods, appearing in bundle-like groups and having pointed ends. Colonies containing these different types were transplanted to brain medium, but growth was always doubtful if not negative, and no attempt was made at purification.

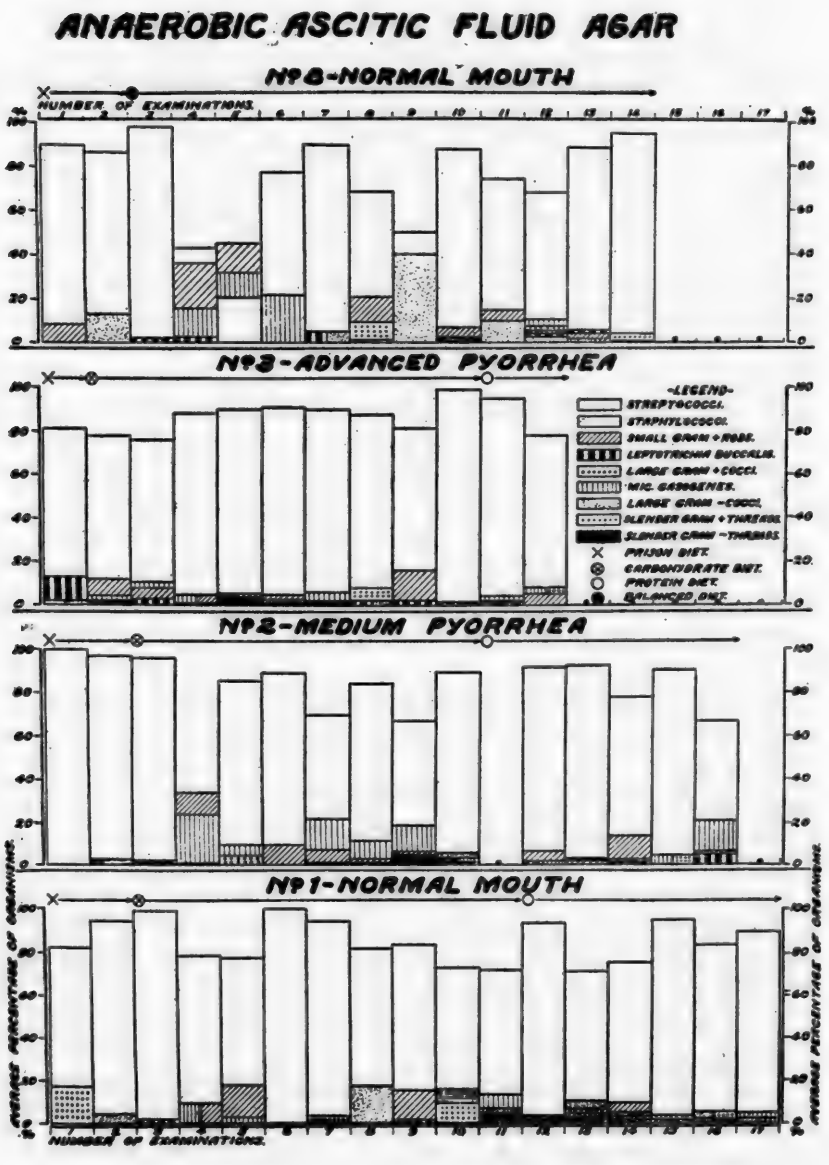


CHART 4a. No cultures were made for No. 2 on the eleventh examination.

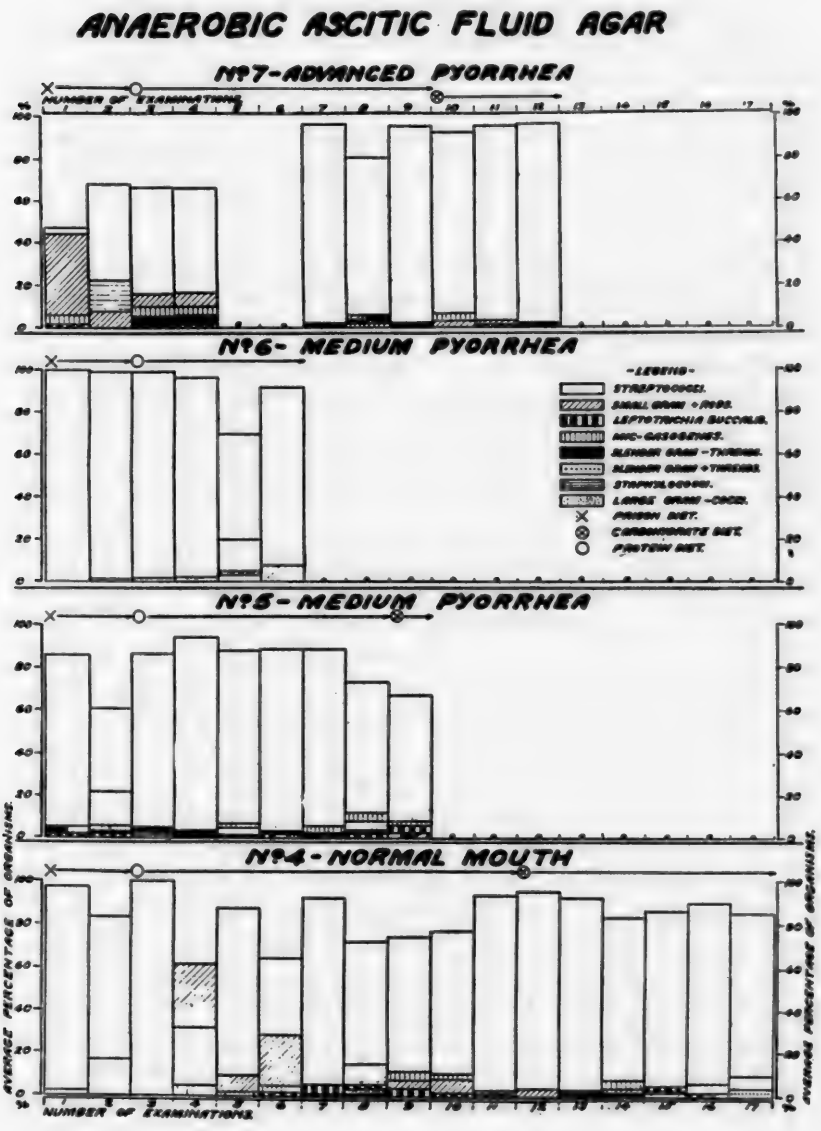

ANAEROBIC ASCITIC FLUID AGAR
No 7 - ADVANCED PYORRHEA
No 6 - MEDIUM PYORRHEA
No 5 - MEDIUM PYORRHEA
No 4 - NORMAL MOUTH
NUMBER OF EXAMINATIONS
AVERAGE PERCENTAGE OF ORGANISMS

CHART 4b. No cultures were made for No. 7 on the fifth and sixth examinations.

Staphylococci and the large gram-negative diplococci of the Flavus group were occasionally encountered on these plates. No sporulating anaerobes were ever found. Kligler and Gies (1915) report having only twice isolated anaerobic sporulating rods from either healthy or decayed teeth, while Hall (1925) working with saliva cultures found spore-forming anaerobes present in only six out of fifty-five samples of saliva. Evidently if they are found at all, it is only as accidental contaminants of the mouth.

Charts 4a and *4b* for Group I, and *6* for Group II, give one a graphic idea of the average percentage plate-incidence, per individual at each examination, of the organisms found on the anaerobic ascitic-fluid agar-plates at dilutions of 1–10,000 and 1–100,000. *Table 13* shows the average percentage incidence of the chief organisms in this medium for each person in the two groups, arranged according to the different diets: the prison food, the carbohydrate, the protein, and the balanced diets. The men in Group II were not examined before going on the diets. No. 8 was the only man on the balanced fare.

Streptococcus is predominant in both groups of men, while the other organisms fluctuate very irregularly regardless of diet or mouth condition. It was thought that the non-fermenters such as *Micro. gasogenes*, *Lepto. buccalis*, and the other thread forms might increase on the protein diet, but this did not occur. In a few instances (No. 4 and No. 7) there was a slight increase of thread forms on the protein fare (*Chart 4b*) and a diminution after the change in diet. In most cases, however, the men show a corresponding number of these organisms on both diets. In *Table 13* the total averages are about the same for the different forms regardless of diet, except perhaps a slight tendency for an increase of *Micro. gasogenes* on the carbohydrate fare.

The normal men on all these diets seem to show the most variations in bacterial incidence, especially No. 8 on the balanced meals. The percentage incidence of organisms given in his chart (*4b*) fluctuates more radically than that for any of the other men. *Micro. gasogenes* is present in large numbers for three successive times and then disappears, to reappear later in a small percentage, while the gram-positive rods and the gram-negative diplococci predominate on several occasions. The thread forms also fluctuate. Kligler and Gies (1915) also noted this variation in their results from normal mouths.

TABLE 13

Average percentage incidence of the organisms on the ascitic-fluid agar plates

GROUP	CASE NO.	PRISON DIET						CARBOHYDRATE DIET						PROTEIN DIET						BALANCED DIET					
		No. of exam.	Av. % strep.	Av. % lepto. buccalis	Av. % mic. gasog.	Av. % small gram + rods	Av. % slender threads	No. of exam.	Av. % strep.	Av. % lepto. buccalis	Av. % mic. gasog.	Av. % small gram + rods	Av. % slender threads	No. of exam.	Av. % strep.	Av. % lepto. buccalis	Av. % mic. gasog.	Av. % small gram + rods	Av. % slender threads	No. of exam.	Av. % strep.	Av. % lepto. buccalis	Av. % mic. gasog.	Av. % small gram + rods	Av. % slender threads
I	1	2	88.5	0	0	0	0	9	85.1	0.12	3.3	5.1	1.7	6	85.3	0.36	3.4	3.4	3.8						
	2	2	98.6	0	0	0	0	8	77.4	0.46	11.5	7.6	0.8	5	84.6	1.21	6.1	5.7	1.2						
	3	1	81.1	13.3	2.2	2.2	0	9	86.9	0.97	2.9	6.1	1.5	2	86.9	0.10	5.4	3.5	0						
	4	2	90.6	0	0	0	0	6	88.5	1.40	4.7	2.3	1.5	9	67.2	2.60	4.3	13.7	0.4						
	5	2	73.8	1.5	5.2	12.1	2.8	1	77.3	6.4	8.2	2.3	2.8	6	75.3	1.20	4.9	3.2	1.4						
	6	2	99.6	0	0.37	0	0							4	89.5	0	1.4	5.4	0						
	7	2	58.1	0.8	3.0	26.0	0	3	93.5	0	3.2	2.3	0.9	5	81.3	0.38	7.3		1.7						
	8	2	88.9	0	0	4.5	0													12	72.0	1.6	7.1	11.2	1.8
Total average....			84.9	1.9	1.34	5.5	0.3		84.7	1.55	5.6	4.2	1.5		81.4	0.83	4.6	5.8	1.2		72.0	1.6	7.1	11.2	1.8
II	9							2	83.3	0.2	9.8	0	6.6	2	88.3	1.10	5.9	3.2	3.0						
	10							2	90.6	0.05	4.5	2.6	2.0	1	83.2	0.30	0	16.5	4.1						
	11							2	58.4	0	29.0	12.5	0	1	94.2	0.80	0.2	2.0	1.7						
	12							2	93.3	2.14	3.09	0.1	0.8	1	85.4	0	4.0	9.5	1.1						
	13							2	92.9	0	3.1	3.3	0.3												
	14							3	89.7	2.2	7.8	2.2	1.2												
	15							1	66.6	3.9	0	29.5		3	79.9	0.5	5.5	8.8	5.0						
	16							1	65.4	0	0	10.6	12.0	2	75.0	4.5	1.8	10.2	9.8						
	17							2	84.7	0.5	6.2	4.9	4.1	2	82.3	2.0	2.1	9.5	0						
	18													4	81.8	1.0	0.6	0	4.9						
Total average....									80.5	0.99	7.05	7.3	3.0		83.7	1.2	2.5	7.4	3.7						

Leptotrichia colonies and other thread forms were found on some occasions on plates from each of the men, with the exception of cultures from No. 7 (protein) regardless of diet or of mouth condition. *Micro. gasogenes* was also present with great frequency.

(*c*) *Anaerobic acid agar-plates* (*pH 4.8–5.0*). The organisms growing in the extreme acid agar were mainly two forms, streptococci and gram-positive, non-motile, non-sporulating, facultative anaerobic rods of the Lactobacillus group. In many instances the aciduric streptococci were difficult to isolate, and were not always classified. Those that were isolated often fell into the non-green producing or gamma group, and frequently fermented inulin. *Strep. mitis* was the only true alpha type found (*Tables 2–4*).

The gram-positive rods usually formed either small, white, opaque, compact, entire colonies with a rough surface, the sand-grain type of Kulp and Rettger (1924), or very large colonies of the same general form, only more creamy in color and with more extended projections (*Plate 1, figs. 4, 5*). They were also finely granular. Sometimes the colonies were very large and complex with thick, opaque, wing-like extensions radiating in several directions. The semi-solid medium used was probably conducive to the large size of these colonies. A known culture of *Lactobacillus acidophilus* gave the same type of large bunchy colonies as above, intermixed with many small compact ones; a few filamentous or x-type of colonies (Rettger and Horton, 1914) were occasionally noticed. These aciduric rods often showed rather thick, comma-like, curved forms when grown in the anaerobic acid medium, but upon transplantation to aerobic blood agar-slants (pH 7.4) they straightened out, becoming more rod-like. Often they showed clubbed endings or were granular and streptococcal in appearance. While initial isolation was easier from acid agar and under anaerobic conditions, yet the rods also grew well aerobically on glucose blood agar-slants. They produced brown discoloration of the blood, together with a granular confluent growth on the surface, and a thick creamy mass at the butt of the slant. They could therefore be differentiated macroscopically from the streptococci and the diphtheroid rods, the latter nearly always forming discrete, whitish colonies, entire, and without much alteration of the color of the blood. Brom-cresol-purple milk was usually strongly fermented, with formation of

acid and clot but no gas. Much acid was produced in many of the carbohydrate media, usually between pH 3.9 and 4.4 in glucose broth. However, not all the isolated strains fermented the same media, but could be tentatively classed into seven different groups according to their carbohydrate reactions as shown in *Table 14*. These groups (types) were given Roman numerals for convenient designation. All of these organisms fermented glucose and maltose; nearly all, lactose and sucrose. The variations were chiefly in the action on mannite, raffinose, and dextrin, presenting many different combinations. All formed acid in brom-cresol-purple milk, and nearly all strains produced a clot without whey in from 24 to 48 hours. The strains of Type II,

TABLE 14

Aciduric rods classed according to carbohydrate reactions (Groups I and II)*

GLUCOSE	MALTOSE	LACTOSE	SUCROSE	RAFFINOSE	MANNITE	DEXTRIN	MILK	TYPE	NO. OF ISOLATIONS IN GROUPS I AND II	PER CENT OF TOTAL NUMBER ISOLATED
+	+	+	+	+	0	0	AC	I	24	31.1
+	+	0	+	0	0	0	A	II	6	7.7
+	+	+	+	+	+	0	AC	III	7	9.0
+	+	+	+	0	+	0	AC	IV	13	16.7
+	+	+	+	0	+	+	AC	V	13	16 7
+	+	+	0	0	+	0	AC	VI	11	14.2
+	+	+	+	+	0	+	AC	VII	3	3.8
								Total	77	

* + = Acid. 0 = No acid. AC = Acid and clot.

however, were slow to attack this medium, and it was often several weeks before a clot was completely formed.

Table 15 gives the number of isolations of the various strains and the percentage of each for the individuals of Group I. While each man often harbored more than one strain, yet certain of these reappeared in the individual on more than one occasion. No. 3, for example, shows 9 or 75 percent of Type I in twelve isolations of the aciduric rods and only two other types, while No. 8 has 8 or 75 percent of Type VI in twelve isolations. Usually one or perhaps two types seem to be characteristic for each individual. The largest total number of isolations of one type was 20 or 30.7 per cent in 65 isolations for Type I. Groups IV, V, and VI came next in nearly equal amounts, and then

the others with smaller percentages. Of the total of 77 aciduric strains isolated from both groups (*Table 14*), Types I, IV, V, and VI gave the highest percentages, 31.1, 16.7, 16.7, and 14.2, respectively. The known strain of *Lacto. acidophilus*, used as a comparative control for these mouth organisms, corresponded biochemically to Type VI, forming no acid from sucrose, raffinose, or dextrin. The morphology and the general cultural characteristics were also similar. Work is now in progress to ascertain the serological relationships of these

TABLE 15

Incidence of the types of aciduric rods in Group I

CASE NO.	TOTAL NO. ACIDURIC RODS ISOLATED	TYPE I		TYPE II		TYPE III		TYPE IV		TYPE V		TYPE VI		TYPE VII	
		No. isolated	Per cent of isolations	No. isolated	Per cent of isolations	No. isolated	Per cent of isolations	No. isolated	Per cent of isolations	No. isolated	Per cent of isolations	No. isolated	Per cent of isolations	No. isolated	Per cent of isolations
1	10							7	70			3	30		
2	13	1	7.6	1	7.6	6	46.1	1	7.6	3	23.0			1	7.6
3	12	9	75.0					1	8.3	2	16.6				
4	8	4	50.0	4	50.0										
6	6	1	16.6					2	33.3	3	50.0				
7	4	3	75.0	1	25.0										
8	12	2	16.6			1	8.3					8	66.6	1	8.3
Total	65	20		6		7		11		8		11		2	
Av. per cent of total no. isolated.....			30.7		9.2		10.7		16.9		12.3		16.9		3.0

different types; to see if the strains from each person are as heterogeneous serologically as they are culturally, or if there is a specificity of one type for the individual. Work of this kind has been done by McIntosh, James, and Lazarus-Barlow (1922), but not extensively for the same individuals over an extended period of time.

The percentage incidence of the organisms from the acid medium is indicated graphically in *Charts 5a*, *5b*, and *6*, for the two groups respectively. In Group I the graphs for the men with normal mouths show the greatest variations in kinds of organism, streptococci and

thread forms intermixing with aciduric rods. There is apparently no relationship with the diet, streptococci from No. 1 having been obtained as often on the carbohydrate diet as on the protein, with a corresponding diminution of gram-positive rods. One notices in these charts that, excepting No. 1, the men with the initial carbohydrate diet have nearly 100 per cent aciduric rods at each examination, even after the reversal of diet. However, with the other men having an initial protein diet there is more irregularity, and more thread forms are present (*Chart 5b*). The threads were found from all the men in this squad while on the prison fare, and did not reappear in this medium until several weeks later. They are then found not only when the men are on the protein fare but after it has been changed (Nos. 4, 5 and 7.) The only time they occur from the first squad on these plates is with No. 3, after changing to the protein diet. Observations with Nos. 3 and 5 and also with Group II were not continued long enough, after the reversal of diets, to afford very definite conclusions. Kligler and Gies (1915) found these thread forms associated in large numbers with the Lactobacillus rods in carious enamel deposits. They must also be strongly acid-enduring to withstand the low pH of the medium from which they were isolated in this work and also the low pH produced by the aciduric rods, although they themselves ferment only a few carbohydrates.

As previously mentioned, the actual number of organisms obtained from the acid agar-plates seems to be greater in the material from the men on the carbohydrate diet than on the protein. Of the species found on these plates, a greater relative percentage of aciduric rods is present when the carbohydrate fare is given, especially in the men with pyorrhea, while streptococci or negative results are more frequently encountered from the protein squad. However, the aciduric rods are also quite prominent in the latter group. Since the men in Group I were originally given their choice of the diets, possibly those who already favored carbohydrates had their natural tendency accentuated, and at reversal of diet, the same organisms having become well established, continued to flourish on the small amount of carbohydrate in that particular diet regardless of its high protein content. Or again, since the protein meals were planned to give acid body-reactions, possibly the effect was sufficiently general to alter the local tissue reac-

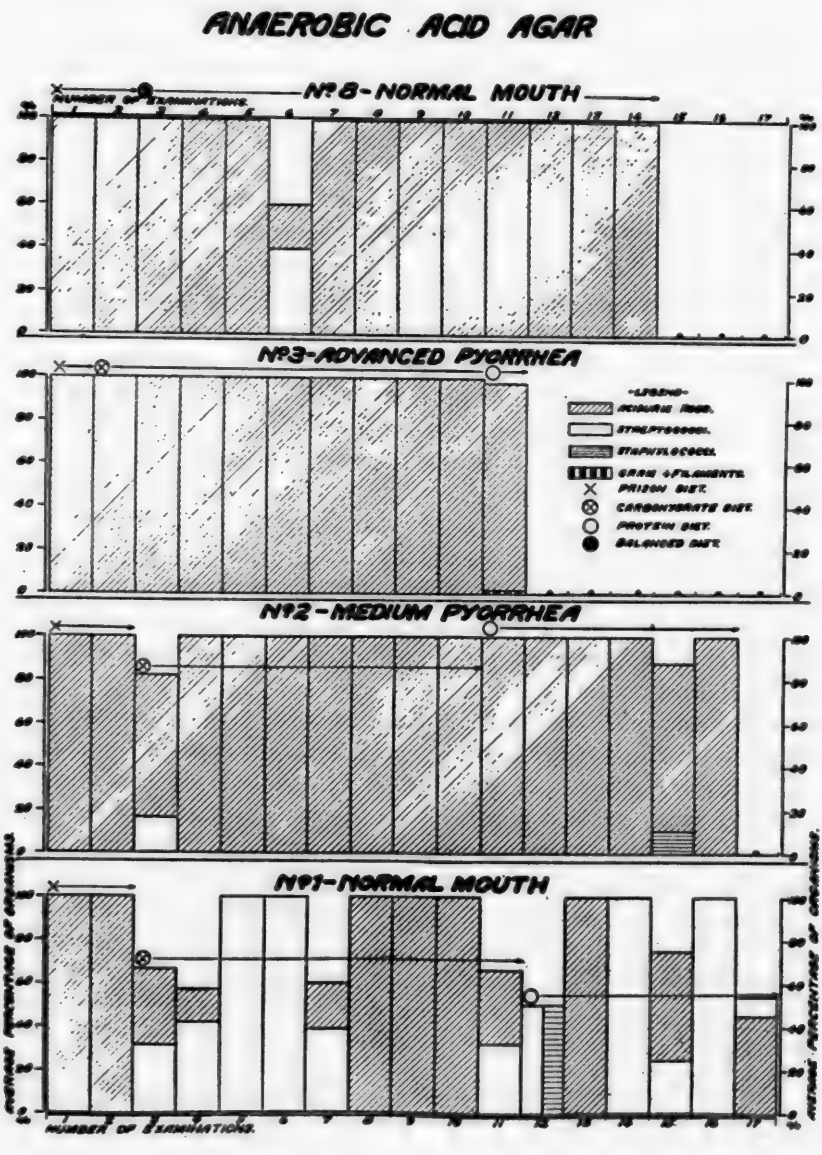

ANAEROBIC ACID AGAR
No 8 - NORMAL MOUTH
No 3 - ADVANCED PYORRHEA
No 2 - MEDIUM PYORRHEA
No 1 - NORMAL MOUTH
NUMBER OF EXAMINATIONS.
PRISON DIET.
CARBOHYDRATE DIET.
PROTEIN DIET.
BALANCED DIET.

CHART 5B

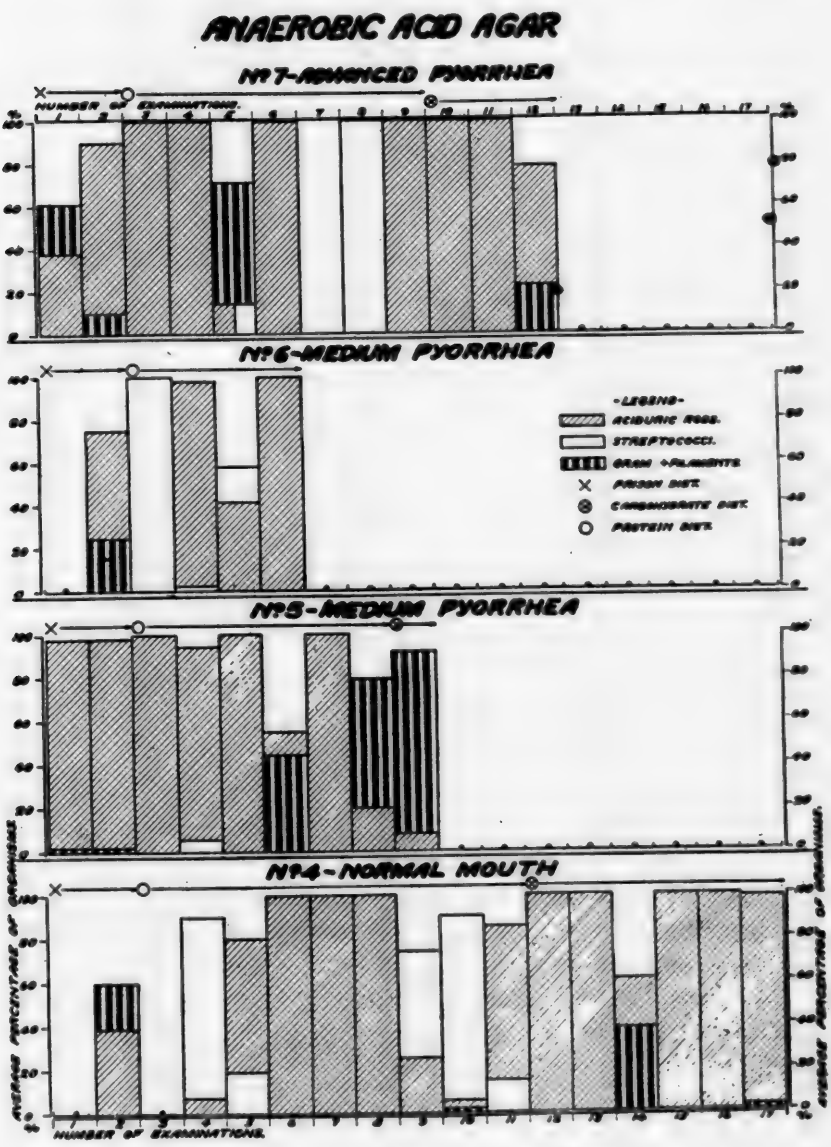


CHART 5b. No cultures were made for No. 4 on the first and third examinations, No. 6 on the first, and No. 7 on the seventh.

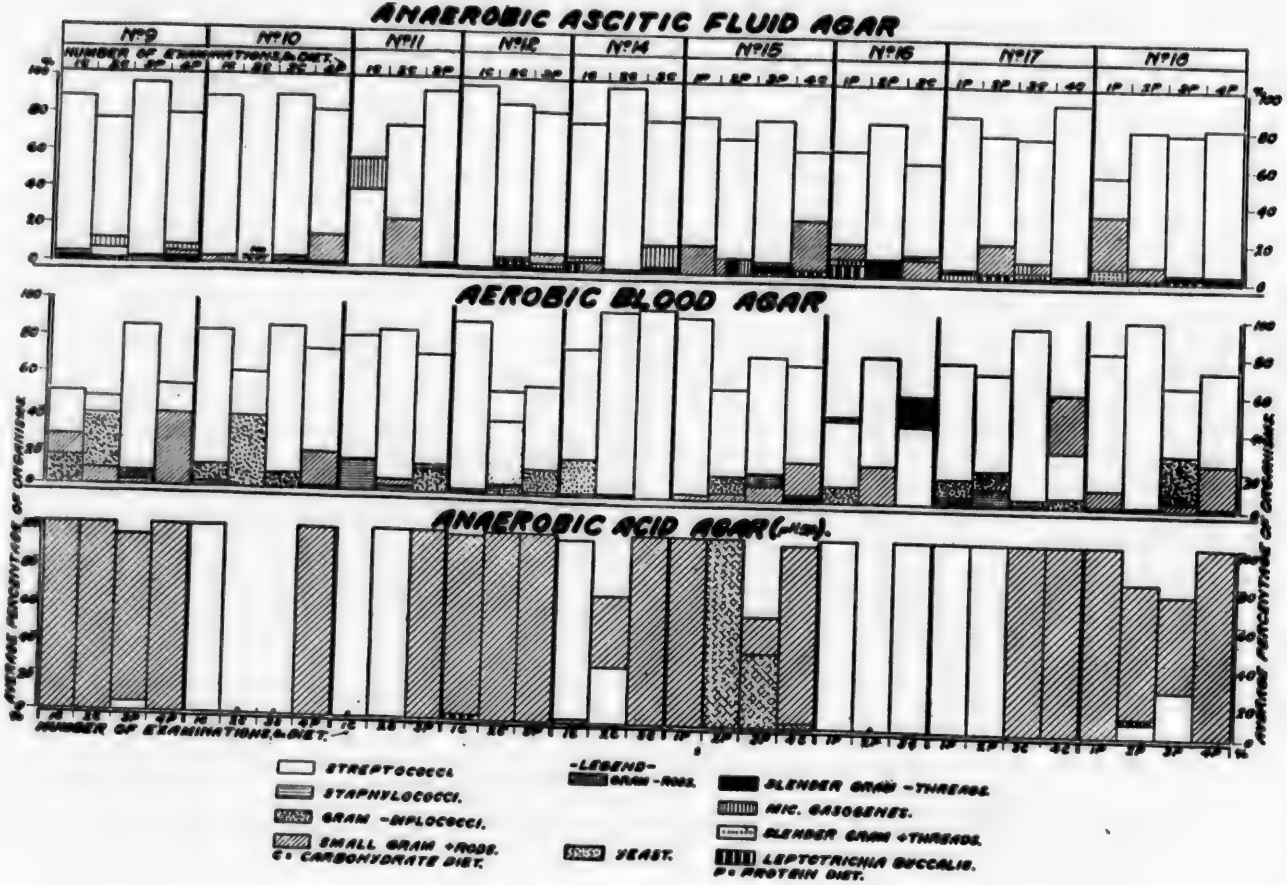


CHART 6. The zero mark indicates that the cultures were negative

tions in the mouth. Examination of the pH values for the saliva, however (*Tables 2–4*), does not uphold this supposition at reversal of diet, nor does it hold when one considers the graphs for the men with the initial protein fare. If the oral tissues had an acid reaction, why, instead of an interspersing of streptococci or an absence of organisms, were not the aciduric rods 100 percent?

The presence of different strains of the Lactobacillus group around the gingival margins of these eighteen men seems of some interest, especially when one considers the recent tendency to associate these organisms with dental caries rather than with pyorrhea. In the present work various strains of aciduric rods were found on one or more occasions from all of the eighteen men, regardless of whether they were normal controls or had different stages of pyorrhea. In most instances these organisms were present at nearly every weekly examination, often covering a period of several months. In comparison with the large amounts of streptococci found on every occasion, their absolute numbers are relatively small; yet, considering their highly fermentive qualities, their mere presence in the mouth may have some significance.

Many authors have ascribed great importance to members of this group in the bacteriology of dental caries. Kligler and Gies (1915) found a large increase in the percentage of *Lactobacillus acidophilus* in the material from early stages of caries. Howe and Hatch (1917) and McIntosh, James, and Lazarus-Barlow (1922) consider the aciduric forms to be of prime importance, if not the etiological factor in caries. Bunting and Palmerlee (1925) found them present in 100 per cent of initial caries and in 94 per cent of advanced caries, while they were isolated from only 16 per cent of non-carious mouths. In a recent paper Bunting *et al.* (1928) offer further evidence to support the theory that these organisms are of etiological significance in dental caries, finding a close correlation between their presence or absence and the dental condition in a survey of 1335 children.

On examining the dental records of the men from both groups, it was found that the amount of dental caries present was negligible except for about three men. Of the latter, one man (No. 6) had two cavities, four incipient pit-and-fissure cavities, and seven incipient gingival cavities; one (No. 4) had five incipient pit-and-fissure cavities and twelve incipient gingival cavities; the third (No. 2) had four defi-

nite cavities, six pit-and-fissure cavities, and nine incipient gingival cavities. Two of these men (Nos. 2 and 6) had medium pyorrhea of the inflammatory type, while the third (No. 4) was a normal control. Aciduric rods were isolated from all of them, although very irregularly from Nos. 4 and 6 (*Chart 5b*). In fact the acid agar-cultures from No. 4 showed the fewest numbers of these organisms of any from the men in Group I. Six of seventeen men (Nos. 3, 5, 7, 10, 16 and 18) showed no caries. Of these, four had medium or advanced pyorrhea, and two were normal controls. Aciduric rods were isolated from all of them. Of the other men, three (Nos. 11, 12, and 15), of whom two were pyorrhetic, had only the smallest signs of dental caries, showing from one to two incipient pit-and-fissure cavities and about the same number of gingival cavities, all inactive. Three (Nos. 1, 8 and 17), of whom only one (No. 17) had a small degree of pyorrhea, showed a few small cavities. Two (Nos. 9 and 14) showed a slightly more advanced condition of dental caries. Both of the latter men also had advanced pyorrhea of the non-inflammatory type.

While it is apparent that incipient caries is present among a number of these men, yet a greater number of those having pyorrhea fall into the groups showing the least amount of dental caries. As mentioned previously, organisms of the Lactobacillus group were isolated on one or more occasions from every one of the men, regardless of whether they had caries or pyorrhea, or both of these conditions. In view of the fact that dental caries and pyorrhea are not generally considered to be coincident in the same mouth to any large extent, and since Lactobacillus rods have been reported so frequently from carious teeth, one would not expect to find the rods so often in cases of pyorrhea. If these forms in the mouth are influenced, as are similar strains in the intestines, by the diet, in the sense that the diet may alter the nature of the pabulum around the teeth, perhaps by encouraging their growth one might bring about a condition antagonistic to whatever may cause pyorrhea, if the bacterial flora is of any significance in this disease.

3. Spirochete count

Since no attempt was made to grow spirochetes, the only gauge of their relative number in the mouth was by counting the organisms on a stained slide. Different forms of spirochete were not differ-

entiated, although most of them were of the open, shallow-spiral type (Spironema). The average number per field, counting twenty fields, was used to calculate the percentage among the total number of organisms in the same fields. *Table 16* gives the average percentage incidence of spirochetes for both Groups I and II, arranged according to diets, where it may be seen that the average percentage of spirals does not vary considerably for the men on the different diets in either

TABLE 16

Average percentage incidence of the spirochetes for Groups I and II

GROUP	CASE NO.	NO. EXAM.	PRISON DIET	NO. EXAM.	CARBOHYDRATE DIET	NO. EXAM.	PROTEIN DIET	NO. EXAM.	BALANCED DIET
			per cent		*per cent*		*per cent*		*per cent*
I	1	2	2.0	9	2.0	6	3.2		
	2	2	10.2	8	2.4	6	5.5		
	3	1	4.5	9	4.7	2	9.5		
	4	2	9.4	6	2.8	9	4.0		
	5	2	1.2	1	5.7	6	5.1		
	6	2	14.1			4	7.2		
	7	2	8.0	3	5.6	7	5.8		
	8	2	0					12	0.79
	Total	15	6.1	36	3.8	40	5.7	12	0.79
II	9			2	5.6	2	4.8		
	10			3	3.0	1	2.0		
	11			2	1.7	1	2.4		
	12			2	6.1	1	9.6		
	13			2	0.4				
	14			3	7.6				
	15			1	0	3	2.3		
	16			1	0.3	2	9.2		
	17			2	4.2	2	4.0		
	18					4	11.5		
	Total			18	3.2	16	4.4		

group, although some individuals show a slight increase on the protein fare. The total averages give a slightly higher percentage for this diet. The normal control on the balanced diet harbored only a very small number of these organisms. Hudson and Parr (1924), working on the relationship between diet and intestinal flora, found that the intestinal spirochetes in rats were also increased on a high protein diet.

It is interesting to note that the average percentage incidence of spirals for both groups is about twice as much for the men with pyorrhea as for those with normal mouths, as shown in *Table 17*. This corresponds with the fact that the amounts of material from the former type of men is much greater than from the latter. Whether there is any other significance is hard to say without obtaining the organisms in pure culture. One of the control men (No. 8) showed a very small number of spirochetes over a period of many weeks. If only a few examinations had been made, it might have been concluded that spirochetes were practically absent in his mouth. On the other hand, one of the pyorrhetic men (No. 15, Group II) also gave a very low

TABLE 17

Average percentage of spirochetes

MOUTH CONDITION	GROUP I		GROUP II		TOTAL
	No. of men	Average per cent spirochetes	No. of men	Average per cent spirochetes	Average per cent groups I and II
Normal	3	3.06	3	2.5	2.78
Pyorrhetic	5	6.6	7	5.6	6.1

spirochetal percentage, and then only when on the protein diet. The work of Kritschevsky and Seguin (1924) lends support to those who favor the spirochetal etiology of pyorrhea alveolaris, since they have been able to demonstrate these organisms in the tissues together with the fusiform bacilli.

4. Salivary pH determinations

The salivary pH of each man was determined at every weekly examination. The saliva was collected under paraffin stimulation into flasks containing paraffin oil to prevent loss of CO_2, while the pH was determined colorimetrically shortly after collection. The pH values for the saliva obtained under artificial stimulation in this manner are high. The figures in *Tables 2, 3, 4,* and *18* may seem higher than those often quoted. *Table 18* gives the average salivary pH values for the men in Groups I and II arranged for each according to the number of examinations on the different diets. The total averages for the 137

examinations for the two groups run about the same for the men on all the diets (pH 7.2–7.4) except for a slightly lower value for No. 8, the control on the balanced diet. There seems to be no lower average value for the men on the protein diet, in spite of its acid tendency, indicating that the salivary reaction is not easily influenced by dietary measures. Variations in the salivary pH seem to be more individual

TABLE 18

Average salivary pH values for Groups I and II

GROUP	CASE NO.	PRISON DIET		CARBOHYDRATE DIET		PROTEIN DIET		BALANCED DIET	
		No. exam.	Av. pH	No. exam.	Av. pH	No. exam.	Av. pH	No. exam.	Av. pH
I	1	2	7.6	9	7.6	6	7.5		
	2	2	7.4	8	7.2	6	7.1		
	3	1	7.4	9	7.2	2	7.1		
	4	2	7.4	6	7.3	9	7.5		
	5	2	7.2	1	6.4	6	7.1		
	6	2	7.2			4	7.2		
	7	2	7.9	3	7.6	7	7.8		
	8	2	7.2					12	7.0
	Total	15	7.4	36	7.2	40	7.3	12	7.0
II	9			2	7.3	2	6.6		
	10			3	7.4	1	7.7		
	11			2	7.3	1	7.6		
	12			2	7.0	1	7.4		
	13			2	7.3				
	14			3	7.2				
	15			1	7.1	3	6.9		
	16			1	7.7	2	7.3		
	17			2	7.7	2	7.4		
	18					4	7.2		
	Total			18	7.3	16	7.2		

than related to any general systemic change, certain men having high or low values rather consistently. No. 7, for example, runs a high pH, while that for No. 15 is low, in spite of the fact that both had pyorrhea and were longer on the protein diet than on the carbohydrate. Other workers have also found that the salivary reaction is not easily influenced by ingested materials. Bloomfield and Huck

(1920) report that the oral reaction is only temporarily altered, and that internal administration of acid or alkali does not influence the salivary pH. Carlson and McKinistry (1924) found that although there is a temporary change in reaction while the material remains in the mouth, the "mouth acidity or alkalinity can not be controlled by any induced substances regardless of its original strength or H or OH ion concentration." Bunting, Nickerson, *et al.* (1928) also did not find any correlation between salivary pH and the diet in their work on dental caries.

VII. DISCUSSION

Both the quantitative and the relative bacteriological findings for the two groups of men, inflammatory and non-inflammatory types (Simonton, 1927) seem to parallel each other in most respects, so far as could be learned from the small number of examinations made for the second group. This would imply that the organisms capable of being cultured from the high dilutions used are practically the same for the two types of pyorrhea alveolaris. Since pyorrhetic "pockets" were also associated with bone resorption in the mouths of many of the Group II men, although bone involvement was a negligible factor with Group I, it seems reasonable to assume that similar organisms would be found in the "pockets" of both types of pyorrhea.

The effect of the dietary changes was about the same for both groups when placed on the same fare. Therefore, in discussing the results of these dietary experiments, no particular distinction will be made between the two groups.

There seems to be a general uniformity in the amount of material removed from around the selected eight teeth of each individual, and also from the teeth of individuals grouped according to similar mouth conditions, resulting in a classification of the men into three groups; normal, medium pyorrhetic, and advanced pyorrhetic. A change in diet does not appreciably alter the average amount from each individual, although there is a slight increase on the carbohydrate diet; a total average of 64.9 mgm. for the men on this diet and 58.7 mgm. for those on the protein fare.

The curves for the weekly amounts of debris obtained have the same general appearance for most of the men, except that those for the

pyorrhetic cases represent larger amounts of material and those for the normal controls show more irregularity of outline. The graphs representing the number of organisms per mgm. obtained from aerobic and anaerobic agar plates, respectively, having the ordinary reaction (pH 7.4), also follow one another quite uniformly. There are no particular differences at reversal of diet.

Streptococci comprise by far the largest group, both relatively and absolutely, when one considers the relative proportion of the different bacteria from the two media. Gram-negative diplococci of the Flavus group I, and diphtheroid rods, 81.8 percent of Type IX, are mixed with streptococci on the aerobic plates, while *Mic. gasogenes*, small gram-positive rods, *Leptotrichia buccalis* and other thread forms, are found in varying percentages in the anaerobic medium. Streptococci, diphtheroids, and Flavus organisms are all strong acid producers. *Mic. gasogenes* and some of the thread forms (fusiform types) are non-acid producers, while *Lepto. buccalis* ferments only glucose.

There is no particular correlation between diet change and the percentage of these different forms, although there may be a slight average increase of *Mic. gasogenes* on the carbohydrate fare. The average percentages of *Mic. gasogenes*, *Lepto. buccalis*, and the slender thread forms on the anaerobic plates, seem about the same for all the men on both diets. The greatest observed variations are among the normal controls, whose charts show first a predominance of one or another form at the expense of the streptococci, regardless of the fare.

Alpha or green-producing streptococci far outnumber all other forms in this group, both relatively and absolutely, in the men of both Group I and Group II. Of 91 examinations, 71.2 percent of all the different organisms were alpha streptococci, 1.4 percent gamma (anhemolytic) and 0.13 percent beta (hemolytic). The alpha forms were classified according to the carbohydrate reactions into several types and subtypes (Brown-Holman classification). Of 197 isolated strains from Group I, there was an average of 56 or 25.7 percent of *S. salivarius* (2.2 and 2.1), 90 or 46.9 percent of *S. mitis* (1.2 and 1.1), 28 or 14 percent of inulin fermenters, and 23 or 11 percent of non-lactose fermenters. Of 78 isolated strains from Group II, 27 or 35.5 percent were *S. salivarius* (2.2 and 2.1), 25 or 32.9 percent were *S. mitis* (1.2 and 1.1), 11 or 14.1 percent were inulin fermenters, and 15 or 17.8 percent failed

to ferment lactose. *S. mitis*, therefore, predominated in Group I, while *S. salivarius* was more frequent among the second group. *S. fecalis* was never isolated from either group. These findings for alpha streptococci are corroborated by the work of Valentine (1927) on focal infections, although she does not report any inulin-fermenting forms except for gamma streptococci.

The alpha streptococci were also divided by colony appearance into three forms, "rosette," "wedge," and "lenticular." Of these three types, 51.6 percent were "rosette," 13.2 percent "wedge," and 33.6 percent "lenticular." No apparent correlation has been found, so far, between colony appearance and carbohydrate reactions. Most of the alpha streptococci were pathogenic for mice. Of the 70 animals inoculated intraperitoneally with 1 cc. each of a glucose broth-culture, 57 or 81.4 percent died within three days. Two-thirds of these were dead in less than eighteen hours. Glynn and Digby (1923) do not report so high a mortality in mice from alpha-streptococci strains isolated from human saliva (60 percent).

The very small incidence of beta streptococci, confined to only a few individuals, both pyorrhetic and non-pyorrhetic, indicates that these organisms are not of great significance in pyorrhea alveolaris. The isolated forms were classed by their carbohydrate reactions into only two types, *S. equi* and *S. pyogenes*.

The graphs for the number of organisms per mgm. from the anaerobic acid (pH 4.8–5.0) agar-plates show more definite results than these from the other two media. The total average number of organisms per mgm. from this medium is much lower than from the others, while the curves for the same reach higher peaks for the men on the carbohydrate diet than for those on the protein fare. When the diet is changed, the curves also change. The average number of aciduric forms appear to be from four to six times greater after the carbohydrate fare than after the protein.

The organisms isolated from the anaerobic acid (pH 4.8–5.0) agar-plates are largely members of the *Lactobacillus acidophilus* group, with very heterogeneous biochemical reactions. Streptococci, thin gram-positive threads, and an occasional yeast organism are the only other species encountered. These are all strongly aciduric forms, and are able to withstand a highly acid medium. Lactobacillus

rods are of themselves capable of producing much acid, as are also streptococci. The thread forms, probably belonging to the aciduric group, are only slight acid producers, yet are evidently capable of existing in a medium of very low pH. Lactobacillus rods were isolated from all of the men, both normal and pyorrhetic, with and without caries, although less frequently from those on the protein diet. They were differentiated into seven separate groups by their carbohydrate reactions and given Roman numerals for convenient designation.

Sporulating anaerobes were never encountered from any of these men, although spore-forming aerobes were occasionally present in small amounts.

The average spirochete count from stained slides showed a very slight increase in percentage for the men on the protein diet. It was also noticed that the average percentage was about twice as much for the pyorrhetic men as for the non-pyorrhetic. Undue significance, however, should not be attached to these observations, since the method of estimation was subject to errors. Nevertheless, it seems quite possible that the spirochetes may be increased in a protein medium and reduced when the latter is reversed, because of their marked protein requirements under artificial cultivation.

The contrasted diets used in this experiment were neither strictly protein nor strictly carbohydrate. However, since each contained enough of the predominant type to affect the urinary pH, and thus differentiate the men in the two squads, they were sufficiently overbalanced in one direction or the other to warrant certain inferences from the information obtained during the six months period. The men were on at least one diet long enough for effects on their general metabolic reactions, which would be evident in case the bacterial flora are influenced by such endogenous factors. The evidence obtained in this work, however, the data from another experiment in which different types of food were given to one man over weekly periods (Howitt, Fleming, and Simonton, 1928), and the fact that the average salivary pH varied very little in any group, seem to show that the oral bacteria are more influenced by the food remnants in the mouth and by oral hygiene than by general systemic reactions.

The evidence obtained from individuals having varying degrees of pyorrhea, and also from normal controls, indicates that the mouth of

any person may harbor a variety of organisms in symbiotic relationships. Some are able to produce much acid, others to endure an extreme acidity although they themselves are non-fermenters, while others prefer a more alkaline reaction and the presence of proteolytic products. It has been shown that aciduric forms are quantitatively increased if the diet is predominantly carbohydrate. However, while a diet having less carbohydrate and more protein may bring about a diminution in the number of aciduric forms, yet apparently there is not a corresponding increase in the proteolytic or neutral types. A quantitative method for spirochete cultivation might give much information in this regard.

This failure to inhibit entirely the growth of acid-forming types may be attributed to the well-known protein-sparing action of carbohydrate. In the presence of only a minute quantity of carbohydrate, acidific organisms induce fermentation before they attack associated protein. Since it was easier to remove the protein food from the carbohydrate diet in these experiments than to eliminate all carbohydrate from the protein fare, there was still sufficient fermentable substance to nourish the aciduric types in the mouth. On the other hand, desquamated oral epithelial and leucocytic remains probably furnished food for the protein-desiring forms, such as the spirochetes, even though food remains were negligible. The symbiotic relationship of these mouth organisms was often noted during examination of the cultures. *Mic. gasogenes* especially was nearly always associated even in a single, well-isolated colony, with either streptococci and aciduric rods, or streptococci and gram-negative threads. Although *Mic. gasogenes* is a strict anaerobe, it was often encountered on aerobic poured blood agar-plates, always in combination with alpha streptococci and occasionally some thread forms, but never alone. If such a symbiotic condition may be sustained in an artificial culture medium, undoubtedly the conditions in the mouth are advantageous for the existence of an extensive and varied flora.

VIII. CONCLUSIONS

1. There was a certain uniformity in the amount of dental debris obtained from each individual and also from the individuals classed according to mouth conditions, these amounts following the groups

as to whether the men had normal mouths, or medium or advanced pyorrhea alveolaris.

2. A change of diet did not appreciably alter the average amount of material from each individual.

3. The average number of organisms per mgm. from aerobic and anaerobic agar-plates having a pH of 7.4 averaged about the same for all the men on all the diets, except for more irregularity in the amounts from the normal controls. The total bacterial counts from the anaerobic acid (pH 4.8–5.0) agar-plates, however, were much lower than from the other two media. But in contrasting the number per mgm. on the different diets, it is seen that the average amounts were greater for the men on the carbohydrate fare than on the protein. For the men of Group I the aciduric organisms were from four to six times as numerous on the former diet as on the latter.

4. Streptococci far outnumbered all other bacterial species in either of the pH 7.4 media, both relatively and absolutely. Alpha or green-producing streptococci were predominant, averaging 71.2 percent of all the aerobic organisms. Gamma types were 1.4 percent; beta, only 0.13 percent.

The alpha streptococci were divided into several sub-groups. *S. salivarius* (2.2 and 2.1) and *S. mitis* (1.2 and 1.1) showed the highest percentages, with a smaller number of inulin fermenters and of non-lactose fermenters. The few beta forms were strains of *S. equi* and *S. pyogenes*.

5. The principal organisms isolated from the extremely acid agar (pH 4.8–5.0) were members of the Lactobacillus group, with a smaller percentage of streptococci and of slender thread forms. The aciduric rods were a heterogeneous group that could be differentiated into seven different strains by their carbohydrate reactions.

6. No sporulating anaerobes were isolated, although spore-forming aerobes were occasionally present.

7. The salivary pH remained fairly constant for the individual and did not seem to vary appreciably with the diet, the average value for all the men on both diets being pH 7.2–7.4. The salivary pH did not follow that of the urine, which was noticeably lower on the protein diet (average 5.7) and correspondingly higher on the carbohydrate fare (average 6.4).

8. Spirochetes were found on slides from all the men, with only a slight increase in percentage for the men on the protein diet. About twice as many of these organisms were found, however, from the men having pyorrhea as from those with normal mouths.

9. The experimental evidence indicates that the oral bacteria are more influenced by the local food remains than by any general systemic effect produced by dietary changes.

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EXPLANATION OF PLATE I

FIG. 1. Gram-positive diphtheroid rods from a glucose blood agar-slant. ×450.

FIG. 2. Pleomorphic gram-positive rods originally isolated from an anaerobic ascitic fluid agar-plate. Preparation from a subculture on an aerobic blood agar-slant. ×450.

FIG. 3. Gram-positive rods originally isolated from an anaerobic acid agar-plate. Preparation from an aerobic blood agar-slant. ×450.

FIGS. 4–5. Colonies of aciduric rods from anaerobic acid (pH 4.8–5.0) agar-plates. ×16.

FIG. 6. Colony of *Leptotrichia buccalis* from an anaerobic ascitic-fluid agar-plate. ×16.

FIG. 7. Alpha streptococcus colony of the "wedge" type from a blood agar-plate. ×16.

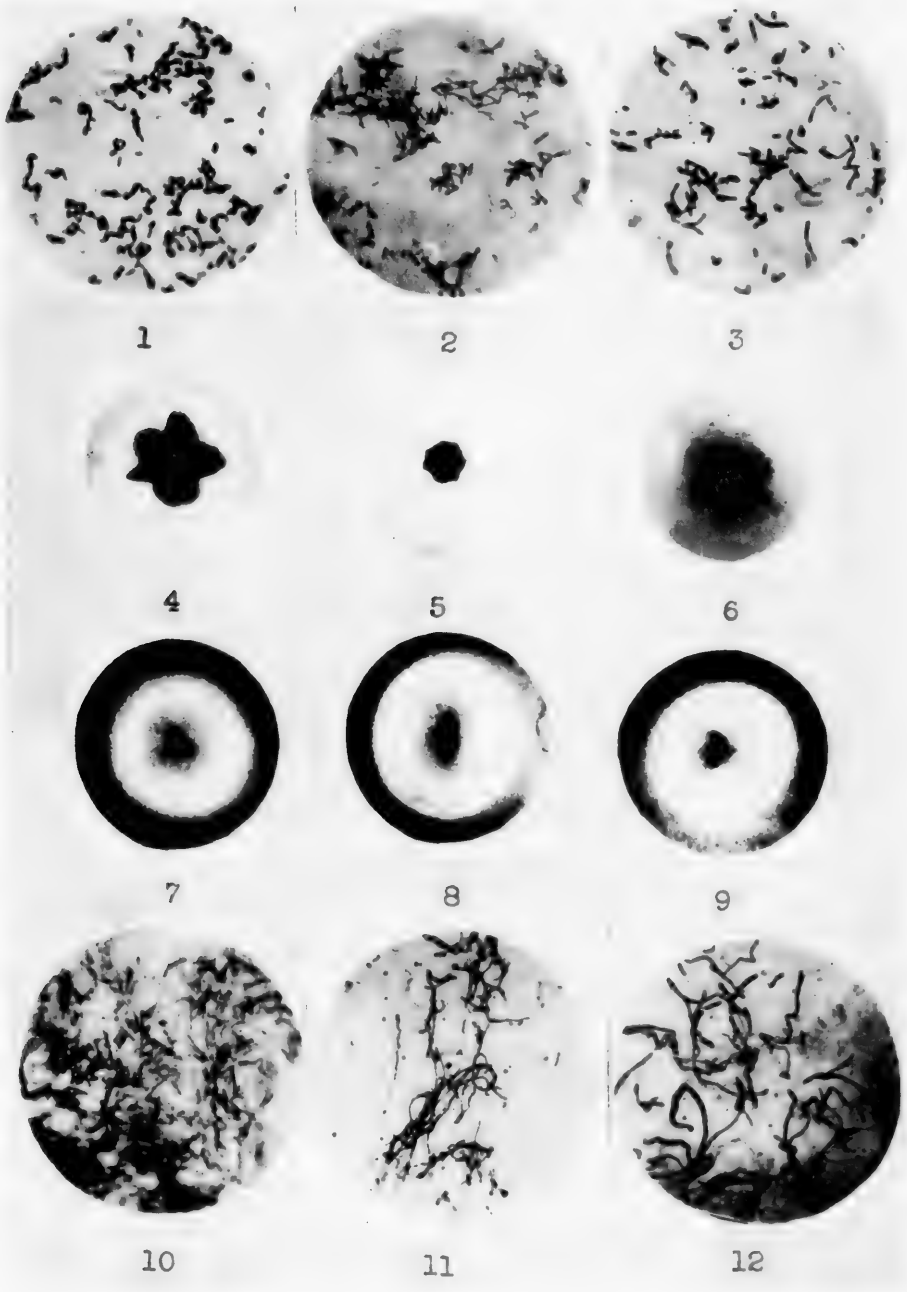
FIG. 8. Alpha streptococcus colony of the "lenticular" or "smooth" type from a blood agar-plate. ×16.

FIG. 9. Alpha streptococcus colony of the "rosette" type from a blood agar-plate. ×16.

FIG. 10. Gram-stained preparation of slender gram-negative fusiform rods from a colony on an anaerobic ascitic-fluid agar-plate. Mixed culture. ×500.

FIG. 11. Gram-stained preparation of slender, wavy, gram-negative threads (*B. fusiformis?*) obtained from an anaerobic ascitic-fluid agar-plate. Mixed culture. ×500.

FIG. 12. Gram-stained preparation of *Lepto. buccalis* threads, showing "whip handle" ends. ×500.



(Howitt and Fleming: Mouth organisms: quantitative study)

ORAL MANIFESTATIONS AND TREATMENT OF VINCENT'S INFECTION[1]

A Symposium

THEODORE B. BEUST, D.D.S., M.D., F.A.C.D.; RAYMOND A. ALBRAY, D.D.S.; AND ISADOR HIRSCHFELD, D.D.S.

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I. MICROBIAL PHASE OF THE ORAL MANIFESTATIONS OF VINCENT'S INFECTION[4]

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Smears from a case of Vincent's infection invariably exhibit two common microbial types: fusiform and spirochaete. The presence of these organisms in a lesion is accepted by a large majority of practitioners and diagnostic laboratory men as an indication of Vincent's infection. Such practice is open to objection. Careful bacteriologists are not convinced of the etiologic significance of these organisms. It has long been known that the same organisms are associated, almost without exception, with other and different inflammatory lesions about the mouth. Pyorrhea, traumatisms, noma, all forms of stomatitis, pulmonary abscess, fatal intestinal necrosis, sinusitis and dental abscess may be attended by their invasion. It is likewise known that vast numbers of similar organisms are present in the oral cavity of subjects who are apparently healthy and, what interests us

[1] Presented at a meeting of the Section of Pathodontia, First District Dental Society, State of New York, at the New York Academy of Medicine, October 24, 1929.

[2] Published in full in the *Bulletin of the First District Dental Society, State of New York*, 1930, x, p. 3.

[3] Published in full in the *Bulletin of the First District Dental Society, State of New York*, 1930, x, p. 7.

[4] This work was supported, in part, by a grant from the Research Commission of the American Dental Association.

here tonight, in whom the syndrome complex of Vincent's infection is lacking. These facts demand discussion. But before giving especial attention to the Vincent organisms, a few words should be devoted to other members of the oral flora.

In relatively healthy mouths the mass of the film is composed principally of Miller's *Bacillus buccalis maximus* (1), *Vincentini's Leptothrix racemosa* (2), and Miller's *Iodococcus vaginatus*. With the advent of an acute stomatitis, however, other organisms quickly gain

FIG. 1. *Bacillus buccalis* and other film organisms

supremacy, an optimum of growth being reached by the *Spirillum sputigenum* (Miller), the large earthworm-shaped *Spirocheta buccalis* (Miller), and the fusiform bacilli and micrococci. The *Spiril. sputigenum* is not unlike the fusiform bacillus; in fact, where the specimens are very large, it is not possible, in the present state of our knowledge, to differentiate them. The habitat of *Spiril. sputigenum* is in putrid pockets, putrid sockets, putrid pulps. Where the secretions of the mouth have reached some degree of putrescence, it may invade the entire buccal cavity. Constantly associated with these organisms

are typical fusiform bacilli. The features differentiating these two types should be made the object of special research. The typical oral flora offer an almost untrodden field for research.

In oral inflammations the large earthworm-shaped spirochete (*Spiro. buccalis*) undergoes active proliferation, the background of the microscopic field being literally carpeted by these organisms in many of the smears. As this form stains only indistinctly with the stains ordinarily

FIG. 2. *Leptothrix racemosa* Vincentini (fragments of fructifying heads). This organism is well represented in the film.

used, the enormous numbers present are not always noticed. Complete staining may be accomplished by the continued application of hot fuchsin, or intensively with Haidenhain's iron haematoxylin. This long known and very common oral inhabitant is often erroneously called Vincent's spirochaete. Its ubiquitous occurrence in oral inflammations of all kinds disqualifies it as an organism of importance in the diagnosis of Vincent's infection. The buccal spirochetes, since Miller's time, have been variously segregated into types. Cahn has

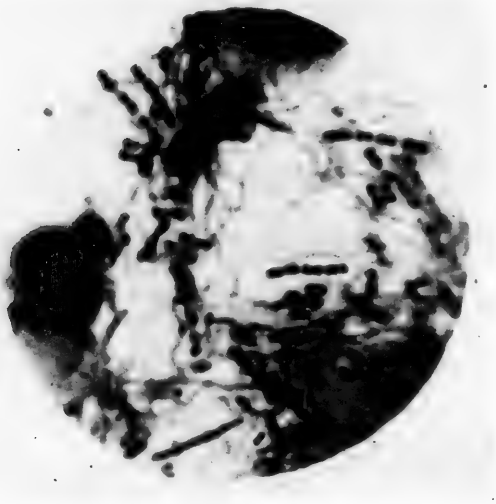

FIG. 3. *Iodococcus vaginatus* Miller. The sheath surrounding the coccus-like bodies has been rendered invisible by the clearing agent.

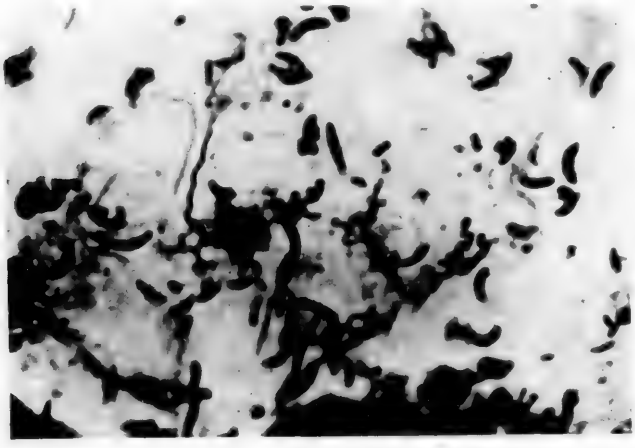

FIG. 4. *Spirillum sputigenum* Miller, from a broken-down root

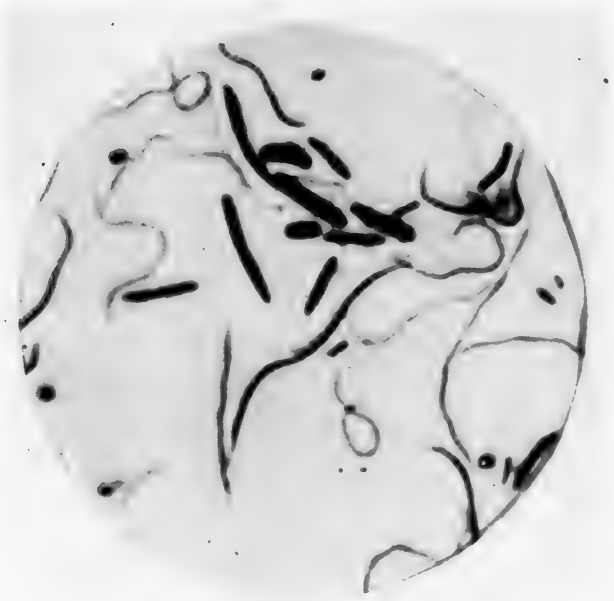

FIG. 5. Fusiform organisms and spirochetes, from a mucus-membrane lesion in a case of Vincent's infection.

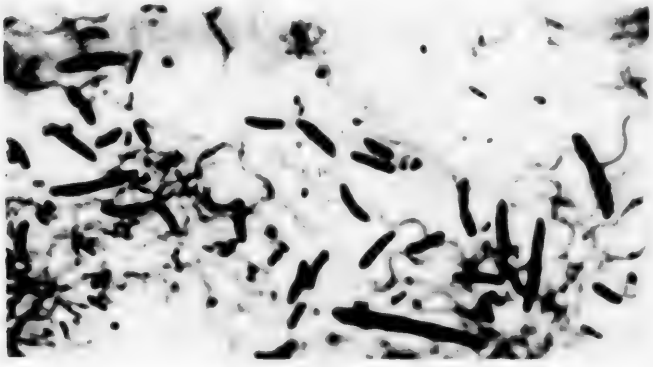

FIG. 6. Fusiform organisms and spirochetes from a pyorrhea case (boy)

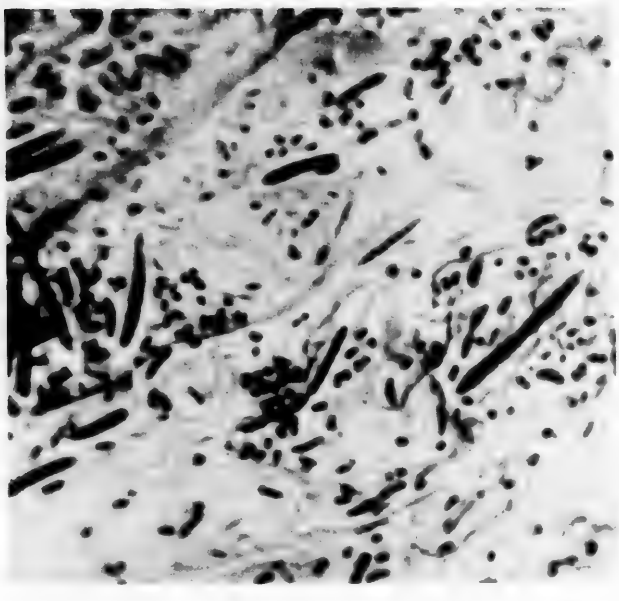

FIG. 7. Fusiform organisms and spirochetes from a pyorrhea case (adult)

FIG. 8. Spirochetes and a fusiform organism from a normal gingival crevice. (Oil immersion 1/12; aper., 1.30; camera extension, 140 cm.; oc. 8 ×)

contributed largely to our knowledge of some of the forms (3). The identity of the fusiform organisms with one of these forms, as claimed by Tunicliffe, has not been, in my opinion, satisfactorily established. I have never, in my studies of the morphology of these organisms, found anything to indicate that a bacillus may turn into a spirochete.

The fusiform organisms, now commonly known as Vincent's bacilli, are constant inhabitants of the oral cavity. They were described by Miller long before the name Vincent became known to our profession. My interest in these organisms began shortly after Plaut's publication in the *Deutsche Medizinische Wochenschrift*, in 1894. Before the discussion of Vincent's infection had assumed a prominent place on the programs of dental meetings, I described a leptothrix form that bore peritrichally ciliated fusiform conidia (4, 5, 6). These organisms are constant inhabitants of inflamed interdental spaces and untreated pyorrheal pockets resulting from Gottlieb's "Schmutz-pyorrhea." It cannot be denied that these fusiform bodies are morphologically identical with the fusiform organisms in Vincent's infection. The view has been advanced that the fusiform bacilli in pyorrhea may be differentiated from those in Vincent's infection—that the bacilli, in the latter case, are smaller. Other diagnosticians state that, in the most virulent cases of Vincent's infection, the larger organisms prevail. The fact is that large and small organisms intergrade so intimately that size cannot be utilized as a criterion in differentiation.

Matachromatism is another property much used in diagnosis. There can be no doubt that metaplasmic areas occur in all of the fusiform organisms of the mouth, and that it is only a matter of arriving at a proper degree of staining to achieve the variegated appearance. Metachromatism or, if preferred, "metachromism," may result in both the progressive and recessive staining methods, the variegation disappearing if the former is continued too long or in the latter if the differentiation is not continued long enough. Metachromism appears in the organisms from both Vincent and pyorrheal lesions.

The numerical incidence of the organisms is also largely used as a basis for diagnosis. Different smears from the same mouth vary in the number of organisms exhibited. Moreover it is obvious, to any

one who has prepared smears for his own inspection, that slides with thread forms or slides with fusiform organisms and spirochetes may be procured at will from the same patient. It is also possible, by spreading properly collected scrapings from a "filth" pyorrhea, to litter a slide with so-called "Vincent's organisms." The presence of these organisms therefore, without a case history, proves nothing.

It appears from this discussion that the etiological microbial factor in Vincent's infection is not evident. Micrococci have been mentioned in connection with the disease. Cocci are, according to my observation, more conspicuous in Vincent's than in pyorrheal lesions. When examining for cocci, the spores of *Lep. racemosa* may be mistaken for cocci. *Lep. racemosa* spores, however, are more rectangular and less chromophilic for most dyes than the cocci. The *Iodococcus vaginatus* of Miller, when viewed in differentiated preparations, may be mistaken for streptococci. The coccus-like bodies of this form, however, are more irregular and vary considerably in size in the same chain. I have not been able to find chain-forming streptococci in my smears. Of course we find them in our cultures, but the oral film seems to be inimical to chain formation.

Vincent's infection is generally looked upon as a contagious disease. It has been known to affect many of the inmates of dormitories simultaneously. But I have never observed a case of infection of a healthy mouth by a patient suffering from the disease. It is readily conceivable, however, that the transmission of common mouth inhabitants which have become virulent, to a subject under physiological stress, might be the exciting cause of a stomatitis.

A discussion of the microbial phase of Vincent's infection should logically consider the means for the disposal of the microbes. The great lesson taught by the microscope has not, in my estimation, been heeded by the profession. It is not the determination of variety, but the demonstration of the presence of microbes, that is important. We have learned from microscopic examinations that, in this lesion, the interdental spaces and gingival pockets are filled with a teeming mass of virus. Notwithstanding this knowledge it is common practice to handle these cases gingerly—to trust to the defenses of the patient to ward off the onslaught. The treatment given by the average practitioner is practically useless. Superficial applications will never

affect the organisms in the interior of the pockets. These concealed armies are the real offenders. If the best is to be done for the patient, then the activities of these organisms must immediately be suppressed. The hemorrhage incident to an immediate energetic treatment disperses the bacteria and their products, reduces the hyperemia, and affords almost instantaneous relief to the patient. If the pockets are evacuated and left surgically clean, the much feared spread of infection will never occur. Any one capable of treating a pyorrheal pocket can get satisfactory results. Microörganisms that may already have entered the tissues, a condition recently depicted by Cahn (7), will fall a prey to the bodily defenses as a result of the improved tonicity of the parts. The effect will be gratifying to both patient and operator.

ADDENDUM

Treatment of Vincent's Infection[5]

1. Rinse the mouth with a solution of 0.5 gram of potassium permanganate in 1000 cc. of water. Thorough repeated rinsings are indicated throughout the following treatment.

2. Swab all lesions and pockets with a wisp of cotton saturated with iodine tincture diluted with an equal part of 50 per cent alcohol.

3. Remove all soft and hard deposits with suitable instruments. Follow the surgical treatment of each pocket or crevice with thorough and repeated irrigation with aqueous 3 per cent hydrogen peroxide carried into the pockets with an abscess syringe No. 33. The H_2O_2 must effervesce actively during use. It will cleanse the parts mechanically, oxidize the bacterial products, stop the hemorrhage, and act as a pain obtundent. *Caution:* Care is necessary in the use of H_2O_2 where deep sinuses with small openings have resulted from chronic pyorrheal conditions.

4. Repeat treatment No. 2. Rinse the mouth thoroughly as directed in No. 1.

5. Napkin one-quarter of the mouth. Dry all crevices. By means of a cotton-covered broach apply sufficient concentrated silver-nitrate solution to whiten the walls of all crevices and interdental sinuses.

[5] This treatment should not be undertaken unless it can be carried out in detail.

Colony formation will hardly ever occur in a pocket so treated. Extend a similar treatment to the other quarters of the mouth. *N.B.* Repeated applications may cause obstinate discolorations of dentin or cementum. Following the treatment with silver nitrate, conspicuous areas may be painted with iodine solution, which will cause the formation of soluble salts. Superficial stains, visible after recovery, may be removed by painting with iodine and subsequently rubbing the stained areas with cotton holding concentrated aqueous solution of sodium thiosulphate. Solutions of potassium permanganate, substituted for the iodine in this decolorization method, are said to act even more energetically.

After treatment. Water containing one-half teaspoonful of sodium borate to the glass should be used for hourly rinsings. See the patient daily for a few days following the treatment. If all of the pockets and lesions have been reached, one treatment will clear most cases. Wherever inflamed areas appear, the treatment outlined above should be repeated.

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II. SOME OF THE MORE SERIOUS ASPECTS OF VINCENT'S DISEASE[6]

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ABSTRACT

Vincent's disease is a virulent infection causing intense suffering, having possibly serious consequences to the patient, and being communicable to others. It occasionally results fatally in patients having lowered resistance, leukemia, pernicious anemia, and leukopenia. Several cases were cited in illustration.

The disease is characterized by the presence in smears of large

[6] See footnote 2.

numbers of Vincent's spirochete and fusiform bacillus. These organisms not only are present in undoubted cases of the disease, but are not found in perfectly healthy mouths. This opinion is held despite assertions to the contrary by many writers.

The symptoms, often not severe even in acute cases, rapidly diminish as conditions develop into the sub-acute or chronic type, when there is special danger of passing the infection to others. This is evidenced by the spread of the disease in armies, colleges, and hospitals.

In the treatment, salvarsan is used both locally and by intravenous or intramuscular injection, in addition to routine sanitive measures. For local application, salvarsan is used in powder form, packed into interdental spaces and pockets.

III. VINCENT'S INFECTION: ITS CLINICAL ASPECTS AND TREATMENT[7]

Isador Hirschfeld, D.D.S., New York City

ABSTRACT

Recognition of the various types of Vincent's infection is very important. While the classical picture of the acute form is definite and readily distinguishable, there are variations in the number and combination of clinical symptoms, some of which simulate other forms of gingivitis or gingival symptoms of systemic disease. When in doubt, the result of a 24-hour test of anti-Vincent treatment will assist in making a differential diagnosis.

Symptoms of incipient Vincent's infection fall under the following headings. (1) Apparently spontaneous interproximal hemorrhage without abnormal redness of gingiva. (2) Inflamed apices of papillae, which bleed easily but are not tender. (3) Same as 2, plus tenderness, but without sloughing. The stereotyped description of Vincent's infection refers to interproximal destruction, but this is not an invariable symptom.

Vincent's infections may be classified under three headings: Acute, subacute and chronic. These are not invariably distinct entities; cases may merge from one to the other or present conflicting symptoms.

The treatment advocated is not new, but if properly executed gives

[7] See footnote 3.

most satisfactory results. Necrotic gingival tissue is wiped off with a cotton-wrapped toothpick dipped in a 10 per cent solution of neo-arsphenamin in glycerin. The patient uses sodium perborate as a mouth wash—one teaspoonful to three-fourths of a glass of warm water, used every half-hour until otherwise instructed. A cathartic is advised, also rest and light corrective diet. Smoking is prohibited temporarily. A ban is placed on spicy foods and alcohol. Tooth-brushing is discontinued during the acute stage. At the second visit the topical application is repeated: at the third visit all gross deposits of calculus are removed. Brushing is commenced as soon as the gingivitis has been reduced. Occasional visits are required for some time, to induce the patient to keep up correct hygiene and function.

TADPOLES FED PITUITARY EXTRACTS

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The works of Evans and Long (2, 3, 4), Smith and Engle (1, 5), Smith (6, 7, 8, 9), and others on the effects of pituitary feeding, pituitary transplants, and pituitary injections, have made it seem advisable to attempt to influence size, growth, and metamorphosis in tadpoles by the feeding of pituitary extracts. The work of Evans and Long in particular indicates that there are two hormones in the anterior lobe of the pituitary; one a growth principle, and one influencing sex maturity and sex changes. The source and preparation of these extracts has been described by Evans in detail in the Harvey Lectures for 1923–24.

Drawing on this work for one's initial hypothesis, it seems likely that the feeding of the "growth" principle to animals would cause a distinct change in size without metamorphosis; while if metamorphosis and sexual maturity might in any sense be considered as successive steps in the same process, the feeding of the so-called "sex" principle should influence metamorphosis before any marked size-growth occurs. This has been done with tadpoles, which were chosen for this work because Amphibia seem to be the only type of animals that are influenced by the feeding of the pituitary principle (8).

For these studies a considerable number of tadpoles were chosen of the species *Rana sylvatica*. The total number of animals was divided into three groups, each group having been put into a large glass bowl containing about three liters of tap water.[2] At the outset of the experiment there were 39 animals in Group I, which received the anterior-lobe "growth" principle; 42 in Group II receiving the anterior-lobe "sex" principle; and 40 in the control group, which were untreated.

[1] The experimental work was done in the Department of Anatomy, University of Pennsylvania, Philadelphia, Pa.

[2] The water was changed daily.

Conditions of environment and feeding were identical for the three groups. For those receiving the "growth" principle, 1 cc. of the Evans alkaline extract (4) was added to the water daily; those receiving the "sex" principle had 1 cc. of the acetic acid extract (4) instead; while the third, the control group, remained untreated. It is not believed that this type of treatment influenced to any extent the H-ion concentration of the medium in which the animals were kept, but that at the same time the animals were forced constantly to ingest minute quantities of the hormone.

The experiments in each case were continued over a period of two months. The results for this period and this type of treatment were

FIG. 1. REPRESENTATIVES OF EACH GROUP, SHOWING AVERAGE RESULTS
a.—Group I; received "growth" principle.
b.—Group II; received "sex" principle.
c.—Control group; untreated.

almost identical. The average length of the animals at the outset of the experiment was about 11 mm.; they were almost the same size throughout. At the end of the experiment the average length of the animals in Group I (those receiving the "growth" principle) was 44 mm.; of those in Group II (those receiving the "sex" principle), 31 mm.; of the controls, 36 mm. Contrary to expectation, the animals in Group II, receiving the Evans "growth" principle, were also the ones which had metamorphosed most rapidly. Those in Group II, receiving the "sex" principle, had shown no evidence of metamorpho-

sis. Only the beginning of limb buds was apparent in the control group. *Fig. 1* shows the average differences among the three groups of animals, which were almost the same size throughout each group.

Smith (8) notes that tadpoles fed on hypophysis show, late in their normal cycle, a relative acceleration in growth, which he believes is due to extension of the second growth-period beyond the normal. Our findings parallel this so closely, as applied to the animals in Group I while not to either of the other groups, as to indicate that the Evans alkaline principle has the property of removing the growth-inhibiting influence. Metamorphosis would seem to be rather a part of the growth picture than analogous with sexual development.

It is interesting to note that while the mortality rate among the animals in Group I was very high, nearly one-half having died and been removed by the termination of the experiment, relatively few animals died in either of the other two groups.

Inasmuch as so many variables enter into an experiment of this type, it is believed that no conclusions are warranted. However, as the results were so constant, it seems apparent that they indicate a direction for further work.

I wish to express my gratitude to Dr. E. M. K. Geiling, of the Department of Pharmacology of the Johns Hopkins Medical School, for so kindly supplying me with the extracts used.

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INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section[1]
December 5, 1929

BISSELL B. PALMER, D.D.S., F.A.C.D., *Editor*

Meeting held at the New York University College of Dentistry, 209 East 23rd Street, New York City, December 5, 1929. Chairman: Leuman M. Waugh, D.D.S., President of the Section.

Scientific program

I. Correlation of radiographic and histologic findings in residual infection. *John Oppie McCall*, D.D.S., F.A.C.D. 113
II. The importance of a new dental bibliography for research workers. *Bernhard Wolf Weinberger*, D.D.S. 114
III. Engineering principles applied to class 2 cavities. *Finn J. Bronner*, D.D.S. 115
IV. Anatomic study of tooth eruption as related to the plane of occlusion. *Egon Neustadt*, M.U.D. 120
V. A preliminary report on parathyroidectomy and its possible effect on the teeth. *Margaret M. Hoskins*, Ph.D. 121

Abstracts

I. CORRELATION OF RADIOGRAPHIC AND HISTOLOGIC FINDINGS IN RESIDUAL INFECTION

John Oppie McCall, D.D.S., F.A.C.D., College of Dentistry, New York University, New York City

Further studies in residual infection have served to emphasize the importance of this research. Negative histologic findings are occa-

[1] Items on the program which for lack of time could not be presented, but have been reserved for a succeeding meeting, were these: "Relationship between migrating teeth and granulomatous tissue in pyorrheal pockets," by *I. Hirschfeld*; "Concerning faults in dental enamel in the teeth of the American Indian," by *Charles F. Bödecker*; "Preliminary report of studies of the teeth of the Eskimos of North Behring Sea and Arctic Alaska," by *Leuman M. Waugh*; "Demonstration of methods for the separation and identification of protein in enamel," by *Theodor Rosebury* and *William J. Gies*; and "A medico-dental case report (toxemia)," by *Bissell B. Palmer* and *Malcolm W. Carr*.—(*Ed.*)

sionally encountered, but infection is found in a large percentage of cases showing one of the following radiographic indications:

(a) Rarefaction, especially with adjacent condensation. (Not distinctive if there is indication of post-operative loss of bone.)

(b) Circular area showing irregular cancellation, usually somewhat rarefied.

(c) Bulging of the border of the alveolar ridge.

(d) Persistance of the original socket outline.

(e) Indentation of the border of the alveolar ridge. See parenthetical comment in (a).

(f) Condensation, with surrounding zone of rarefaction.

To be considered conclusive, readings should have been made at least one year after extraction.

Histologic findings show two typical tissue reactions in cases of residual infection—usually both are found, but in material recovered at operation only one type may occur. The first is round-cell infiltration, the other fibrosis. These are usually associated. In addition, osteoclastic activity may occasionally be seen. Cases presenting radiographic evidence of condensation also show, under the microscope, typical condensing osteitis.

II. THE IMPORTANCE OF A NEW DENTAL BIBLIOGRAPHY FOR RESEARCH WORKERS

Bernhard Wolf Weinberger, D.D.S., College of Dentistry, New York University, New York City

The last attempt in this country to compile a complete dental bibliography of all dental books was made by C. George Crowley in 1885. In 1891, Alfred Sternfeld and Karl Kellner in Karlsruhe, Germany, carried the work of Crowley a step further. They included not only the dental books, but also the important dental contributions in medical and allied sciences, yet excluded the material in dental journals. All dental bibliographies since 1891 relate to the material found in one library or another. The *Index of the periodical dental literature* (Black) has taken care of the periodical literature, but it includes as a rule only such books as have been reviewed in dental journals. In Germany, Port's *Index der deutschen*

zahnärztlichen Literatur und zahnärztliche Bibliographie is mainly that of the German periodical literature from 1847 to 1914, inclusive. Therefore, the research worker as well as the librarian is handicapped in the use of dental books that have appeared since 1891.

In June, 1929, the First District Dental Society of the State of New York attempted to fill this gap by publishing a bibliography of the material in the dental library of the New York Academy of Medicine and in my own library. Although this bibliography is complete so far as these two libraries are concerned, and perhaps includes the more important dental works, there are thousands of books that do not appear in it. In order to aid those interested, a complete dental bibliography of dental books should be published. To obtain the proper backing, it is desirable for this Association to recommend the preparation and publication of such a bibliography, which should include an author index and a subject index. Before publication, printer's proofs should be sent for correction and revision to all libraries containing dental material mentioned in it. A key number should be given each such library, so that designated books could be readily located.

III. ENGINEERING PRINCIPLES APPLIED TO CLASS 2 CAVITIES

Finn J. Bronner, D.D.S., College of Dentistry, New York University, New York City

The prevalence of caries on proximal surfaces has defied measures from all branches of preventive dentistry, and the corner restoration of a posterior tooth is still the most time-consuming problem confronting the average practitioner. The uncertainty of restorative results has generally been charged to deficiencies and dimensional changes of cast gold and amalgam. Furthermore, the gold necessitates difficult heat controls and greater destruction of tooth-structure; the amalgam requires a manipulative skill above the average. Hence a recheck was made of all preparatory procedures in order to ascertain whether an imperfect technic could be counterbalanced by greater perfection in other steps. Engineering methods were employed and a resurvey was first made of tooth form and material. A great many sections were made in many different planes, and enamel and total

wall-thickness measured as well as size and form of pulp-chamber. See *Table 1.*

Many posterior teeth were subjected to heat, and the enamel removed to determine whether the contour of the dentin followed that of the enamel. A notable exception was found on the buccal side of the lower posterior teeth, where a decided concavity existed in the dentin between the gingival third and the cusp. (This is evidently an adaptation to the occlusion with the maxillary buccal cusps. As it was found in lesser degree in the gorilla, and only in the

TABLE 1

Average total wall-thickness, in mm. from surface to pulp

POSITION	TOOTH	INCISAL EDGE	LINGUAL PIT	PROXIMAL WALLS	CLASS 5 REGION	FROM MESIAL CORNUA TO OCCLUSAL SURFACE; DIRECTION IN LONG AXIS OF TOOTH	DISTANCE BETWEEN THE MESIO-BUCCAL AND THE MESIO-LINGUAL CORNUA	FROM OCCLUSAL SURFACE TO POINT BETWEEN CORNUA
Upper	First incisor	4.7	2.5	2.3	2.6 (min. 2.0)			
"	Second "	4.5	2.2	2.0	2.6 (" 2.0)			
"	Canine	4.7	2.9	2.3	2.9 (" 2.6)			
Lower	First incisor	3.7		1.8				
"	Second "	3.7		1.8				
"	Canine	4.7		2.1				
"	Posterior teeth			2.5	3 (min. 2.5)	3.2–5	3.1	3.2–5

hypoconid, it is probably conditioned by the complex lateral excursion more characteristic of the human mandible).

Resistive and retentive forms were analyzed in view of form and wall thickness. It was found that proximal areas susceptible to caries are determined by the profile of the narrower of the two teeth in contact (gingivally, 4–6 mm.; marginal ridge, 3 mm.). This profile is roughly cone-shaped in ratio 5:3. Hence, outline-form should converge in the same ratio (*fig. 1*). The area between contact point and marginal ridge is *never* the primary seat of decay, and therefore requires no extension for prevention. Thus, the insistence on parallel buccal and lingual outline found no support.

FIG. 1. Converging lines *B* are parallel with tooth outline. Compare the parallel lines *A* with corresponding lines in the dentin (*fig. 3*).

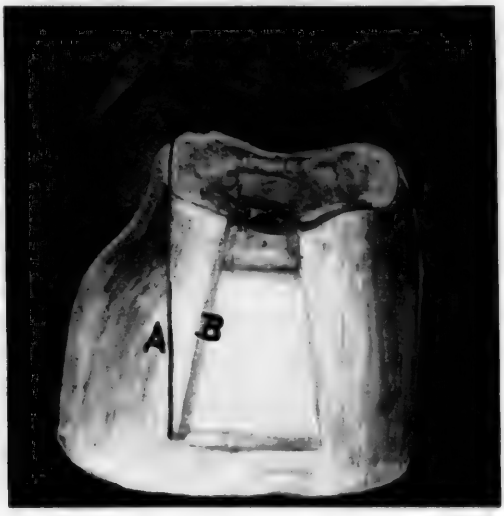

FIG. 2. Double exposure of author's model (25 diam.), showing enamel cap superimposed on the dentin core.

The dentin, the only available "rock" into which to anchor a stress-bearing structure, is still more tapering (ratio 2:1) owing to the 4-times thicker enamel at the level of the proximal marginal ridge. From an engineering point of view parallel walls embracing the susceptible Class 2 area reduce this dentin-cone to unsafe thinness under the cusps. This condition is further aggravated in the lower posterior teeth by the presence of the aforementioned buccal concavity in the dentin (*fig. 2*). Besides, as the mesial cornua contrary to accepted belief are very persistent, even in a receding pulp, such procedure will irritate, expose, or endanger the pulp horns (*figs. 3* and *4*).

FIG. 3

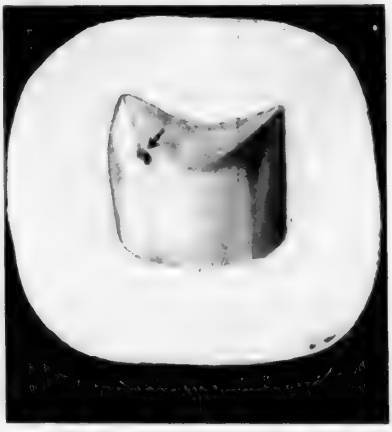

FIG. 4

FIG. 3. Dentin core, showing weaknesses of parallel proximal outline. Note pulp exposure indicated by arrow.

FIG. 4. Dentin core of $\overline{6}|$, showing form of pulp chamber and exposure of M.B. cornu.

Consequently we hereby furnish proof that the surveyed terraine (tooth) demands converging outline and still more converging resistance-form, the dimensions of which are determined by the data submitted. Depth should have minimum safety margin of 1 mm. (from the pulp).

An analysis of the retention-form brought out a grave violation of engineering principles when dealing with materials subject to accidental stress before hardening, and to subsequent slip (flow), chief

among which is amalgam. By inclining the gingival wall this flow is largely counteracted at a point ordinarily difficult of adjustment (the gingival margin). (*Figs. 5* and *6*.) To dislodge the proximal section of a filling built on such inward incline, tipping must take place on the gingival margin as fulcrum. In so doing the deepest point of the incline now encounters resistance against the axial wall, and must move up to the level of the fulcrum before exit can occur. This, however, is prevented by the converging buccal and lingual walls. Thus the proximal section has become *a self-locking device* (same principle in Class 3) with wide application, obviating for

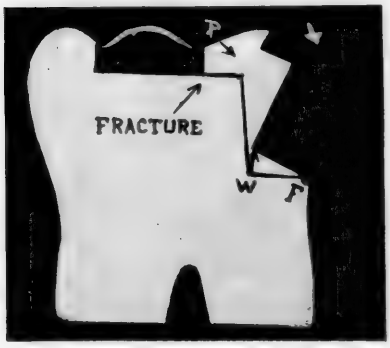

FIG. 5

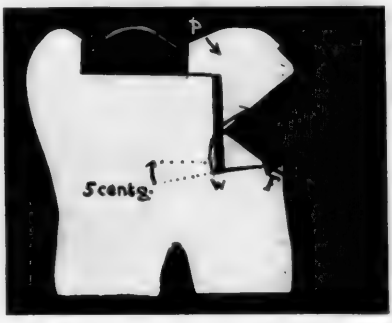

FIG. 6

FIG. 5. Model showing that *W* encounters no resistance to the force *P*. *F* = fulcrum.
FIG. 6. Model showing that the axial wall offers resistance to force *P* when gingival wall is inclined. *F*. = fulcrum.

example the necessity for dove-tailing the occlusal part of a cavity where material is not available. Nevertheless, it should be understood that the occlusal step, which is not illustrated, gives additional retention to the filling.

The form described requires for its execution two enamel hatchets for a mesial cavity, and two additional ones for a distal cavity, with 5-centigrade inclination of the edge. But of these incidentally also replace the four gingival margin trimmers, the net saving is two instruments. For Class 3, the same cavity-form obviates the necessity for incisal retention and saves two instruments.

IV. ANATOMIC STUDY OF TOOTH ERUPTION AS RELATED TO THE PLANE OF OCCLUSION

Egon Neustadt, M.U.D., College of Dentistry, New York University, New York City

The plane of occlusion is determined by two factors: the eruptive power of the teeth and the force of occlusal contact. Under normal conditions, these two forces are in equilibrium: the eruptive power of the teeth, tending to increase the growth of the alveolar process, is checked by the action of the muscles of mastication, which in turn tends to depress the teeth and decrease the height of the alveolar bone. If teeth lack occlusal contact, their eruptive force is demonstrated by an excessive eruption. It must be understood that this eruption consists of a continued growth of the alveolar process and of the periodontal tissues along with the tooth. It should not be confused with that tooth eruption which increases the size of the clinical crown through recession of the periodontal and bone tissues. In the eruption of teeth without occlusal contact, the size of the clinical crown does not change.

We can follow this tooth eruption on molars that have lost their antagonists; on deciduous molars and premolars, where the development of the curve of Spee is dependent upon the conditions under which the newly erupting teeth are provided or not provided with occlusal contact; and on the incisors, where the picture changes slightly and teeth without contact can grow past each other and so produce excessive overbite. Wherever in the mouth the phenomenon of excessive eruption is studied, it is found that teeth always erupt until checked by contact. And if the contact is not provided by opposing teeth, it is finally provided by gum-tissue of the alveolar process in the opposing jaw, by tongue, or by lip. The only exception to continuance of eruption, where teeth are without occlusal contact of any kind, is the hindrance caused by lack of space, either through crowding or a "wedging" influence of adjoining teeth.

The eruption of individual teeth or groups of teeth to a higher occlusal plane very seriously disturbs occlusal balance in centric and excursive positions. For this reason, occlusal contact must be considered just as important as proximate contact. Furthermore,

continued tooth eruption is not limited to the period of youth: it is always present, though it proceeds at different rates according to age and person.

V. A PRELIMINARY REPORT OF PARATHYROIDECTOMY AND ITS POSSIBLE EFFECT ON THE TEETH

Margaret M. Hoskins, Ph.D., College of Dentistry, New York University, New York City

It is well known that the incisors of the white rat break off some time after the animals have been deprived of their parathyroid glands. At the same time the submaxillary gland undergoes a considerable enlargement. It has been stated that neither of these results occurs if the thyroid as well as the parathyroid is removed. It has been found in this laboratory that unusual activity of the thyroid is correlated with exceptionally low mortality from tetania parathyropriva. It has also been observed that parathyroidectomy stimulates the thyroid for a time at least. Among those animals that live long enough to show the dental deficiencies there would, therefore, be a large number which were hyperthyroid at the time of the operation or immediately after it. It is suggested that this hyperthyroidism and the overgrowth of the submaxillary may be important factors in the dental deficiencies of parathyroidectomized rats; and that the condition does not result entirely from the disturbance of the calcium metabolism that follows the operation. Further work on the problem is necessary before a definite conclusion can be reached.

SOME UNUSUAL CASES AT THE INFIRMARY OF THE DENTAL FACULTY OF THE UNIVERSITY OF TORONTO[1]

W. HENRY GODSOE, D.D.S. (*Dal.*), B.Sc. IN DENTISTRY (*Toronto*)

University of Toronto, Toronto, Canada

To the writer's best knowledge, after a superficial survey, there is little or no literature on the type of cases cited below (nos. 1–3). The characteristics which seem to set them apart are these:

(1) The condition has been present from an early age, i.e., from the eruption of the deciduous teeth.

(2) Each patient, excepting the dental abnormality, is apparently healthy, and sound both mentally and physically.

(3) The disease, if such it might be called, while running a chronic course as to time, has been by no means latent. It might well be called *destructive chronic pedo-periodontoclasia*. In each case the teeth dropped out with the eruption of the deciduous teeth.

(4) Each case exhibits an angry, circumscribed, areola of inflammation around the necks of the teeth, in some cases extending even further.

(5) Once a tooth has fallen out, the investing tissues seem to return to a healthy condition, while the process continues around the other teeth.

Case no. 1

About twenty years ago, a messenger boy called at the Dental Infirmary to have his teeth examined. He was about 13 years of age, rather stout and rugged, and rode a bicycle through the snow of mid-winter to deliver telegrams. He told us that teeth fell out from time to time, and that his gums were sore and swollen around the remaining teeth. The family and personal history revealed nothing. Examina-

[1] Read at a meeting of the Toronto Section of the International Association for Dental Research, University of Toronto, May 13, 1929.

tion showed three or four teeth remaining in each jaw, most of which were lying on their sides. The lower permanent cuspids, the only teeth left in the anterior part of the jaw, were quite horizontal; the second molars were completely out of occlusion. In the upper jaw there were left a few badly tipped posterior teeth and one cuspid. The mucous membranes were normal where there were no teeth, but around the teeth were highly inflamed tissues, with a good deal of sloughing, and pus exuding from the gingival trough.

The boy was seen but once or twice, nothing was done for him of any help, so he drifted away and has not been heard of since.

FIG. 1. Case 2. Radiogram showing resorption of the alveolar process; typical for the three K—children.

Case no. 2

Report on the K—family. William, Albert, and Dorothy K— were 14, 13, and 11 years of age, respectively, when they were brought for examination to the Dental College by their mother, who sometime before had taken a rest cure for tuberculosis. *William*, the eldest, had already lost the lower left and right centrals, which had dropped out. There was considerable inflammation around the necks of all the teeth. Although none of the *other children's* teeth had fallen out, yet they all exhibited a similarity in the appearance of the gingival tissues, and the resorption of the alveolar process and pocket formations (*fig. 1*). The year previous to their examination at the Dental College,

they had been going to their school dentist, who apparently saw nothing untoward in their oral condition After six-months' treatment at the Dental College, they were discharged with their mouths in a healthy condition.

Family history. There is a family history of this condition on the father's side as far back as the third generation, i.e., their great-grandmother. Their father lost his teeth, so far as can be ascertained, before he was 20; the grandmother, at an estimated age of between 18 and 20; the great-grandmother, around the age of 26. All the teeth, so far as is known, *fell* out—were not extracted owing to caries, etc. These cases differ from the others in that there is a previous family history.

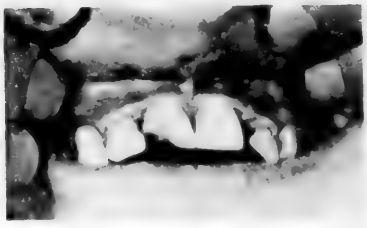

FIG. 2

FIG. 3

FIG. 2. Case 2. Photograph: Dorothy K.—. See *fig. 3*

FIG. 3. Case 2. Photograph: Dorothy K—. See *fig. 2*

Postscript. Two years and a half have elapsed since the K— family has been seen by the Dental College. The writer called on this family a week ago, but was able to see only one of the children, *Dorothy*, whose mouth condition warrants further treatment. Photographs were obtained (*figs. 2* and *3*). The two boys were at work and could not be seen. In a conversation with *William* over the phone, he said that his teeth were worse than his sister's, his brother *Albert* having the best teeth in the family.

Case no. 3

For lack of a more appropriate name, the condition presenting in this case is typical of the term, "chronic destructive pedo-periodonto-clasia." The patient, a girl of 9, was born in Ontario. Excepting the

dental abnormality, she appears to be normal in every way. Color is good, and she seems quite active and healthy. On March 8, 1929, she

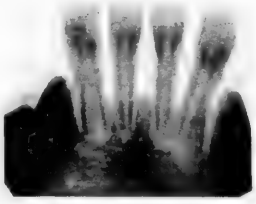

FIG. 4. Case 3. Radiogram: girl at 7

FIG. 5

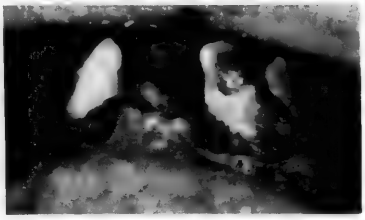

FIG. 6

FIG. 5. Case 3. Photograph, upper: girl at 9. See *fig. 6*
FIG. 6. Case 3. Photograph, lower: girl at 9. See *fig. 5*

FIG. 7

FIG. 8

FIG. 7. Case 3. Radiogram, upper: girl at 9. See *fig. 8*
FIG. 8. Case 3. Radiogram, lower: girl at 9. See *fig. 7*

was brought by her dentist to the Dental College for examination. A consultation was held, but no definite diagnosis was offered, beyond

the conclusion that the child was suffering from a case of chronic pyorrhea, which had been running a course of some five or six years. All were of the opinion that it was primarily a constitutional disturbance. Steps were taken to study this possibility so far as the available facilities would allow (*figs. 4–8*).

When presenting at the Dental College the teeth that are "blocked in," in *fig. 9*, had already fallen out. There were no remaining deciduous teeth. In this connection it is well to remember that the

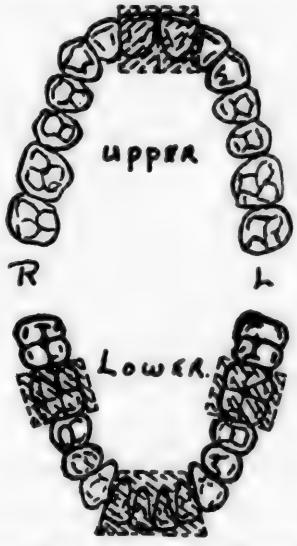

FIG. 9. Case 3. Diagram showing general dental condition. The teeth that are "blocked in" had already fallen out. There were no remaining deciduous teeth. Age of patient, 9.

child is only 9 years old. The four permanent cuspids were slightly loosened, and the lower left lateral was about ready to fall out. (It has since dropped out.) The gingival tissues investing the teeth were highly inflamed, and pus exuded around the gingival margin from time to time, particularly in the left superior anterior region. A full-mouth x-ray showed the two bicuspids on either side of the lower jaw in a process of eruption, the upper already through the gingival tissue. The lower left first bicuspid has since come through

and appears to be quite solid. The alveolar process surrounding the remaining teeth had resorbed considerably, and there were pocket formations around practically every tooth. The data for a *blood count* follow (per cmm.): Red cells, 4,672,000; white cells, 10,200. *Differential count:* poly., 67 percent; neutr., 2 percent; lymph., 27 per cent; mono., 0 percent; eosin, 3 percent; basoph., 1 percent. *Polynuclear count:* Class I, 49; II, 40; III, 9; IV, 2; V, 0. The "red" count is quite normal; the "white" shows a marked leucocytosis; the "differential" indicates nothing unusual. The "polynuclear" count gives a high "left-hand count," which is indicative of a grave systemic condition. Whether this is due to the condition of the mouth, or to an influence which may be predisposing to the oral state, cannot be said. A urinary analysis gave no indications of abnormality.

Case history, by the mother. "The first tooth appeared at ten months. It was the outside one of the four in front at the top. Then two at the bottom and two at the top and so on, until it came to the eye-teeth and stomach-teeth. When seventeen months old, she seemed to take spells, when you would think she was frightened and she had a tendency to back up, and appeared dizzy—her lips would get rather purple in color, she would go to sleep afterwards—sometimes not for long, and again for an hour or so. We saw a doctor at once. She claimed the child was perfect—could find no cause; treated her for worms with no results, but still gave her medicine to take. In about $1\frac{1}{2}$ months her face broke out in big sores which continued, and the spells got worse until she would take four or five in a day. The sores appeared to have a lot of pus in them. When nineteen months old one eye-tooth and one stomach-tooth came through, both within a day or so of each other. Then the spells stopped, and she never took any more, and got the rest of her teeth without any trouble.

"When about $3\frac{1}{2}$ years of age, we noticed her teeth getting loose and gums inflamed, and they would fall out sometimes in the night. We took her to the doctor again, and she gave her medicine; and again her face broke out with the same sores, but this time more on her legs, and some on her arms. She became so weak she could not walk (she started walking at thirteen months), so the doctor sent us to a specialist, Dr. MacGregor at London. He said it was the medicine that was poisoning her system, and he gave us a formula for a different

medicine along with tablets, and the sores soon disappeared. But her teeth still fell out. When 5 years of age she had whooping-cough, when 6 she got her permanent teeth, which have all come out (*fig. 4*). The dentist treated her for pyorrhea, but did no good. When she had this trouble at 3½ years, her ears were very bad and would discharge a lot. She had her tonsils and adenoids removed last July, had measles two months ago. *Diet:* fed from the breast until thirteen months, with some cereal, crusts, and soups after ten months. After weaning, she got as much milk as she would take; also cereal, eggs, vegetables, some fruit when in season. After 2½ years, a little meat. She was kept much to this diet; has been given cod liver oil; always had a good appetite and slept well."

Special study of the case. It was decided that the best place for the child was a hospital, where she was admitted the following day (March 9, 1929). The writer obtained the opinion of one of the physicians at the hospital, which was this: "I can see nothing wrong with the child except that she is suffering from chronic Vincent's infection. There is nothing wrong with her constitutionally." This was rather disappointing, but as there was still some doubt, the case was kept under observation. At the hospital the diagnosis was "chronic Vincent's angina." Although this condition might have been present, the case did not give the usual clinical picture. One observer said: "I have seen a great many cases of Vincent's, but never one that resembled this. The child's condition certainly points further than that." The case was followed up and the child kept under observation because of the peculiarities of her condition.

The patient has been in the hospital for two months. During this time she has had five injections of Novoarsan intravenously. She has also had a local treatment from the dentist consisting of Churchill's iodine and silver nitrate solution twice weekly. In my opinion there is very little change in the local condition. The gingival tissues are not quite as inflamed as formerly, but there is still considerable inflammation surrounding the necks of the remaining teeth, and pus is exuding from the gingival margin of the superior left cuspid. The inferior left lateral has since fallen out. On the 23rd of April another specimen of *blood* was obtained, and *counts* as above were made, with the following results: Red cells, 4,200,000; white cells, 4,833. *Differ-*

ential count; poly., 65 percent; neutr., 3 percent; lymph., 29 percent; mono., 0 percent; eosin., 2 percent; basoph., 1 percent. *Polynuclear count;* Class I, 48; II, 28; III, 20; IV, 4; V, 0 (*fig. 10*).

There is some slight improvement in the general or systemic condi-

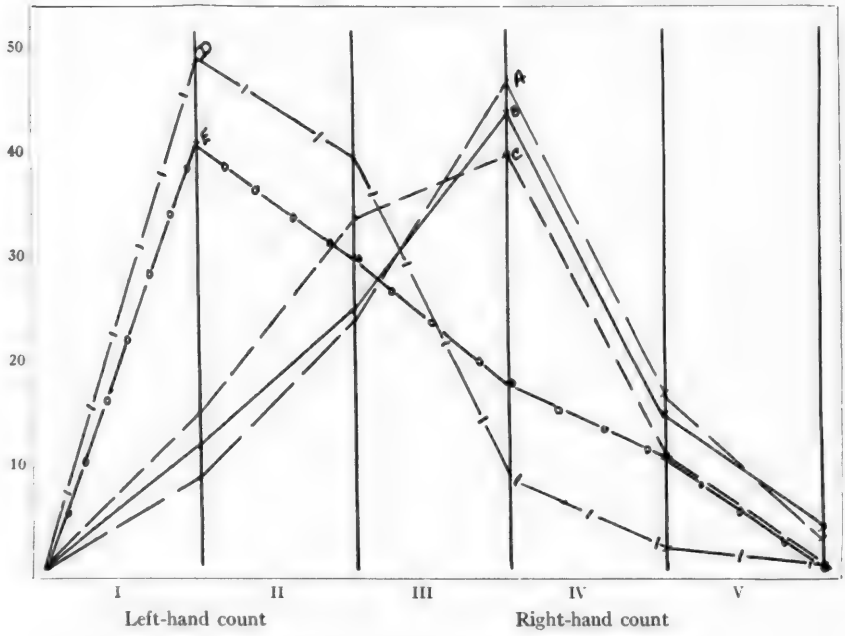

FIG. 10. Case 3. Graph showing details of "polymorphonuclear counts."[2]
D.—Before treatment, on March 8, 1929. *E.*—After treatment, on April 23, 1929.
Normal (highest extreme).....A
Normal (mean).....B
Normal (lowest extreme).....C
Curve (before treatment).....D
Curve (after treatment).....E

tion; but certainly not the improvement that should be expected, if there were only Vincent's angina to contend with.

[2] See "Polynuclear Count:" Cooke and Ponder, 1927. The data indicate the amount of infection present. This is shown by the percentage of cells in Classes I and II, as against that in Classes III, IV, and V, the first two being termed the "left-hand count."

Case no. 4

Child presenting chronic periapical abscess. The patient, 4½ years of age, was brought to the Dental Infirmary, Feb. 16, 1929, suffering from a periapical abcess of the superior right central. The face was so swollen that the eyes were half closed, the swelling extending laterally on either side to the malar bone. The mother stated that a similar swelling occurred five months previously, and that the child had then been taken to a physician, who said it was an infection and lanced it. There was apparent betterment until four days before the patient presented, when the face began to swell again, the child complaining of pain and was feverish. The mother then decided that the teeth might be causing the trouble. The temperature of the patient was 104; the pulse, 120; the respiration, 24. The child was lethargic almost to the point of unconsciousness.

After a consultation it was decided that immediate extraction was necessary, which was done with ethyl chloride as a local anesthetic. About five minutes after the removal of the tooth, the child showed distinct signs of activity as compared with her condition before. There was considerable exudation of pus on extraction. The child was seen two days later. The temperature was 98.3; pulse, 60; respiration, 22.

Quotations Relating to "Dentistry or Stomatology"

THIS JOURNAL uses the term "dentistry" to signify the science and art of oral health service in all its local and systemic relationships. The meanings of words, which are not necessarily restricted to *etymological* significance, acquire expanded or new import in accordance with convenience and usage. Thus the term "medicine," which originally meant to remedy or cure, now also means *to make remedial or curative treatment unnecessary.* "Stomatology" is a synonym for "dentistry." We prefer the older term because its use respects both the history of dentistry and the efforts of those who have brought dentistry to its present status.—*Journal of Dental Research*, 1929, ix, p. iii.

If dentistry were merely dental mechanics, a two-year course in dental technology, based on graduation from a high school and conducted along lines similar to those for the education of optometrists, would probably afford sufficient training for dental practice, and oral health-service could be reserved for a new specialty to include oral surgery, which might be named stomatology and made analogous to ophthalmology and other specialties of medicine. But with dentistry developed into the *full service equivalence* of an oral specialty of medicine, the creation of a medical specialty of stomatology would be redundant—and there would be no occasion to substitute "stomatology" for the name established by custom.—*Carnegie Foundation's Bulletin on Dental Education*, 1926, p. 135.

The assumption that dentistry would be something more or better than it is, if all or a part of it were called "stomatology," exemplifies a readiness to attach more importance to labels than to contents, or more significance to make-believe than to reality. The term "medicine" originally meant to remedy or cure. ["*Medicina* (ars)"—healing art; "*medicina* (res)"—a remedy; "*medicus*"—of healing; also a physician.] Today "medicine," by including measures to *prevent* sickness and by throwing *etymology* overboard, means not only to remedy or cure, but also *quite the contrary*—to make remedial or curative treatment unnecessary. As medical knowledge has advanced and medical procedures have been changed in consonance, including the development of prevention and of surgery, new meanings have been imparted to the term "medicine." But instead of replacing "medicine" by a succession of words intended to indicate *etymologically* the broader or deeper modern meanings, the import of the original term has been conveniently enlarged as the scope of medical practice has expanded. Thus, the term "medicine" has come to mean more and more as *physicians*, without restricting themselves to its *etymology*, have included *more and more in its significance.* Along the same line of reasoning one sees clearly that, although *etymologically* the term "dentistry" refers only to the teeth, nevertheless logically it also comprehends the contiguous parts and their systemic relationships. Certainly by *usage* in conformity with this logic, and also with the criteria of common sense, the term "dentistry" has *acquired* the broader meaning suggested by the more detailed and lengthy term "oral health-service." By logic, usage, and convenience, the term "dentistry" has thus become the *practical* equivalent of "stomatology," which *etymologically* means the sum of scientific knowledge concerning the mouth, but *etymologically does not include the rest of the body* (although "stomatology" is now *used* by some to include systemic relationships with the mouth). This being true, we are unable to agree that "dentistry" and "dentists," which are terms that have long represented the earnest and aspiring endeavors of generations of faithful practitioners of oral health-service, should be formally replaced for *etymological* reasons by "stomatology" and "stomatologists."—*Report of a committee, American College of Dentists*, March, 1929.

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